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Resumen por el autor, A. C. Ivy.
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Estudios experimentales sobre el tallo cerebral.

II. Un estudio comparativo de la relación de la corteza cerebral con el nistagmo vestibular.

El autor ha estudiado la relación de la corteza cerebral con el nistagmo vestibular en la rana, tortuga, paloma, conejo y gatos y perros jóvenes y adultos. Llevó a cabo varias ablaciones del cerebro observando su efecto sobre el nistagmo vestibular, usando como estímulo la rotación. La extracción del cerebro en la rana, tortuga y paloma no perturba el nistagmo vestibular. La extracción completa del cerebro en el conejo con la destrucción extensa del tálamo no suprime el componente rápido del nistagmo, siempre que la temperatura del cuerpo se conserve normal. Las observaciones de F. T. Rogers sobre la reducción de la temperatura del cuerpo subsiguiente a las lesiones del tálamo y su efecto sobre los reflejos ha sido confirmada por el autor en el conejo. En el gato y perro la ablación de la corteza motriz en la región del área ocular causa un aumento temporal, con alguna permanencia, de 5 a 15 veces mayor, en la duración de post-nistagmo, cuando se hace girar al animal sobre el lado de la lesión. Hay un aumento en la reacción del nistagmo cuando la desviación es opuesta al lado de la lesión, con alguna disminución, pero no cesación, cuando la desviación tiene lugar hacia el lado de la lesión. Los hechos apuntados dan validez a la conclusión de que el componente rápido del nistagmo vestibular no se debe a la integridad de un arco reflejo cerebral, sino que depende de algún centro situado debajo del tálamo, sobre el cual ejerce el cerebro una bien reconocida acción inhibitoria.

Translation by José F. Nonidez
Carnegie Institution of Washington

EXPERIMENTAL STUDIES ON THE BRAIN STEM

II. COMPARATIVE STUDY OF THE RELATION OF THE CEREBRAL CORTEX TO VESTIBULAR NYSTAGMUS

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NINE FIGURES

During the course of a study involving a series of cerebral ablations, it was suggested by Dr. F. T. Rogers that observations be made upon the nystagmus reaction. Wilson and Pike ('11, '13, '15) are of the opinion that the quick component of nystagmus is dependent upon the integrity of a cerebral reflex arc. Pike ('17) reports that removal of one cerebral hemisphere abolishes the quick movement when the slow movement of the eyes is directed to the side of the remaining cerebral hemisphere. On the other hand, Bauer and Leidler ('11) report that the quick component of vestibular nystagmus is not dependent upon the cerebral cortex and that "extirpation of the cerebrum, thalamus, even extensive destruction of the midbrain and probably with the inclusion of the oculomotor nucleus does not disturb vestibular nystagmus." Hence there is quite a discrepancy between the reports of the two groups of investigators which has stimulated this study.

METHODS

Frogs, turtles, pigeons, rabbits, kittens, cats, pups, and dogs were studied. All operations upon mammals were done under aseptic conditions. Observations were made before and at different periods following the operation. When the animals were comatose, or markedly depressed, or showed symptoms of increased intracranial pressure, a notation of such a condition was made. Autopsy was done upon every animal and the brain was preserved.

Observations were made upon rotatory and postrotatory nystagmus. The animals were placed upon a turntable and rotated, the speed and number of rotations being controlled.

RESULTS

Frog. Some observers have reported the presence of compensatory movements of the eyes of the frog, others have not. This discrepancy is probably due to the condition of the frog and the acuteness of observation. Out of about one hundred frogs examined only a few were found that did not show on rotation a true vestibular nystagmus with slow and quick components. The small green frog (*Rana pipiens*) and the jumbo bull-frog (*Rana catesbiana*) were used. The latter is much better than the former for study, as the nystagmus reaction is more marked and the eyes are larger. Several precautions are necessary in order to observe the reaction. It is necessary to rotate the frog slowly; if rotated too rapidly, only deviation occurs. The quick component is very slight in degree ($\frac{1}{8}$ to $\frac{1}{32}$ inch, depending on the size of the frog), for the deviation in the frog is not marked. It is easier to observe, if the head is held between the fingers to prevent head nystagmus. If the frog struggles much, it will be absent. Pinning to the frog board often inhibits the reaction. Postrotatory nystagmus is very infrequent. I have observed it, however, consisting only of one or two movements. The temperature of the frog is very important. The best reaction occurs at 18° to 20°C.

Decerebration in the frog never abolishes the quick component of nystagmus nor interferes with the nystagmus reaction in any way. If the frog is depressed as a result of the operation, then deviation only is observed.

Turtle. Wilson and Pike ('15) report that nystagmus is absent in the turtle. My observations are to the contrary, provided the turtle's temperature is between 10° and 39°C. On either side of these temperatures the quick component is abolished and deviation only is present.

This effect of temperature upon nystagmus is only to be expected when it is recalled that reflexes in general are depressed by temperature on either side of the normal or optimum.

A true vestibular nystagmus is present in all species of turtles and terrapins received at our laboratory (*Chrysemys elegans*, *Chrysemys concinna*, *Chelydra serpentina* et al.). From two to six quick movements of the eye occur while rotating the turtle through ninety degrees at a rate of one turn in two seconds. From three to twelve postrotatory quick movements occur when rotated ten times at a rate of one turn in two seconds. There is a latent period of from one to four seconds before postrotatory nystagmus appears. Prince ('17) reports such a latent period in very young animals, which I have confirmed. (This, I have found, is also the case in new-born babies.)

TABLE 1

Showing the effect of temperature upon the quick component of nystagmus in the turtle

TURTLE	QUICK COMPONENT DISAPPEARED AT		OPTIMUM TEMPERATURE
	°C.	°C.	°C.
A	10.0	38.0	12.0-36.0
B	9.0	37.0	13.0-36.0
C	13.0	38.0	18.0-36.0
D	9.5	38.0	16.0-35.0
E	5.5	37.5	10.0-35.0
F	18.0	36.0	32.5-34.0

Deviation occurs at 2°C. Temperature was not reduced lower. Deviation disappears at 39° to 40°C. Head nystagmus appears simultaneous with or shortly after the eye nystagmus. Both head and eye movements occur synchronously and simultaneously.

Hemi-decerebration and total decerebration without injury to the optic lobes, midbrain, and underlying nerves have no effect upon nystagmus in the turtle. If the turtle is depressed as a result of the operation, the number of nystagmic movements is diminished or entirely absent. A decided increase in the number of nystagmic movements has been seen not infrequently. (This increase and the effect of lesions of the optic lobe, reported at the meetings of the American Physiological Society, spring, 1919, will be discussed in a later paper.)

Pigeon. Ewald ('10) reported the presence of true vestibular nystagmus in pigeons. According to Wilson and Pike ('15), it

is present in the pigeon only to a slight and less pronounced degree than in mammals. According to my observations, the average number of rotatory nystagmic movements for thirty pigeons¹ when rotated through an arc of ninety degrees at a speed of one turn in two seconds was six, the minimum number being three, the maximum ten. The average number of postrotatory movements for the same group of pigeons when rotated ten times at a speed of one turn in two seconds was eleven, the minimum being four, the maximum twenty. The average duration of the after-nystagmus was five seconds.

Hemi-decerebration in the pigeon has no effect upon nystagmus.

Complete decerebration with even extensive injury to the thalamus does not abolish the quick component of nystagmus provided the temperature of the bird is kept normal. Rogers ('18) has shown that the temperature of the decerebrate bird with thalamic lesion must be kept normal in order to get normal decerebrate behavior. In two such pigeons, whose body temperature fluctuated with the temperature of the surrounding air, it was found that the quick component of nystagmus disappeared at 34°C. in one and 35°C. in the other, while deviation persisted.

Rabbits. True vestibular nystagmus is present in the rabbit. The average number of rotatory movements for eight rabbits when rotated through an arc of ninety degrees at a speed of one turn in two seconds was five, the minimum being four, the maximum seven. The average number of postrotatory movements for the same group of rabbits when rotated at the same speed was sixteen, the minimum being seven, the maximum twenty-four. The average duration of the after-nystagmus was eight seconds.

Some rabbits show a marked variation in the number of movements and the duration of the after-nystagmus, although they were rotated at the same rate of speed and other factors were controlled. One of the rabbits varied from seven to twenty-two movements, or from four to ten seconds, during the course of

¹ The pigeon's head should be fixed so it cannot be moved. The pigeon has a method of shaking and twisting its head which markedly inhibits the number of the movements and duration of the after-nystagmus.

one examination. Struggling has a marked inhibitory effect upon the duration and the number of movements of the after-nystagmus. When the eye is turned in the direction of the quick component, the nystagmus is increased, and vice versa, as is the case in man. With these and other factors controlled, there was a marked variation in two of the eight rabbits examined.

Hemi-decerebration in the rabbit does not abolish the quick component of nystagmus. It is present when the animal is rotated in both directions, but there is a difference in intensity

TABLE 2

Showing the effect of hemi-decerebration in the rabbit on vestibular nystagmus

RABBIT	NORMAL				LEFT HALF CEREBRUM REMOVED			
	Rotatory		Postrotatory		Rotatory		Postrotatory	
	Rotated to		Rotated to		Rotated to		Rotated to	
	Right	Left	Right	Left	Right	Left	Right	Left
I	5 ²	5	18-22	18-22	0-1 ¹	3-4	18-22	6-12
II	4-5	4-5	10	15	0-1 ¹	5-6	10-20	5-7
III	5	5	12-15	14-18	0-1 ¹	5	18-31	7-11
IV	5	5	14-16	15-18	0-2 ¹	5-7	18-25	8-11

¹ When the table was stopped, having rotated only a quarter of a turn, two to three postrotatory movements invariably occurred. This never occurs in the normal rabbit nor in the hemi-decerebrate when rotated to the left.

² Figures represent the number of quick movements when the rabbit was rotated through an arc of 90° at a speed of one turn in two seconds. The postrotatory movements were elicited by rotating ten times at a speed of one turn in two seconds.

(table 1). The rotatory nystagmus is diminished, almost absent, when rotated opposite to the side of the lesion, but the post-rotatory nystagmus is intensified. The rotatory nystagmus is normal when rotated to the side of the lesion, the postrotatory nystagmus being apparently somewhat diminished. Sometimes a latent period of from two to five seconds occurs before the after-nystagmus, which is liable to cause one to overlook the reaction entirely. If the animal is depressed or manifests marked activity and restlessness, the quick component is irregular in its occurrence or may be entirely absent.

Complete decerebration in the rabbit does not abolish the quick component of nystagmus. The entire thalamus can also be destroyed (figs. 8 and 9) without abolishing the quick component. In the decerebrate rabbit the quick component persists until the animal becomes depressed because of degenerations involving lower centers or because of inanition, it being very difficult to keep these animals in a good state of nutrition. In the decerebrate rabbit with destruction of the thalamus the temperature becomes subnormal and the quick component disappears, but will return again, if the animal is placed in an incubator and its body temperature raised to normal. Deviation still persists with subnormal temperature. In such a rabbit immediately after the operation and for four to five hours later the quick component is very manifest, but after this time it is irregular and subject to wide variations. Two such animals manifested no rotatory quick component when tied to a board, but when held in the hands and rotated the quick component was present. Without taking into consideration these last two points, along with body temperature, one might overlook the presence of the quick component in rabbits without cerebrum and thalamus.

The rabbit is a convenient animal in which to demonstrate the presence or absence of the quick component following various brain lesions.

Cats. Six cats have been worked upon with the same results as observed in the rabbit and the dog.

Kittens and pups. The same observations hold true in young animals as observed in the adult, except for a general rule that the depression from the operation is less marked and the effects produced are more temporary. However, one pup, which was operated at the age of four months and is now one year old (August 1, 1919), in which the left motor cortex, occipital cortex and basal portion of the temporal lobe was extirpated, now shows three postrotatory movements when rotated to the left, and eight when rotated to the right. The increase in the after-nystagmus when rotated opposite to the side of the lesion seems to be permanent in this pup.

Observations upon the time of occurrence of nystagmus in new-born kittens confirm those of Prince ('17). Well-defined vestibular nystagmus does not appear until the end of the second week and the after-nystagmus is preceded by a latent period in kittens and pups. (Observations which will be published later, have been made upon new-born babies.)

Dogs. Rotatory vestibular nystagmus is present in the dog to the same degree as it is in the rabbit. Postrotatory nystagmus, however, is not so marked in the dog as in the rabbit. The average number of postrotatory movements for thirty dogs when rotated ten times at a rate of one turn in two seconds was six, the average time of duration being four seconds. One dog in this series had to be rotated at a rate of one time per second in order to elicit a postrotatory response of four movements. In other words, individual variation occurs in dogs as in rabbits and other animals studied, which necessitates the determination of the normal of the dog before operating.

Ablation of the eye-motor area of the cerebral cortex, also with inclusion of the lateral and basal portions of the temporal lobe, does not abolish the quick component of nystagmus. On the contrary, there is an increase in the rotatory nystagmus when rotated to the side of the lesion and a marked increase in the post-rotatory nystagmus when rotated opposite to the side of the lesion. This increase is more or less temporary, but persists as long as six and eight months (dog V, table 3) in two animals permitted to live that long. This increase consists in a five- to fifteen-fold increase in the number of movements and a two- to four-fold increase in the time of duration of the after-nystagmus. This marked increase only lasts from one to three weeks, after which it reduces gradually to only a two- or three-fold increase. The magnitude of the quick component is decreased, that is the arc through which the eye is moved during the quick component is decreased as compared with the normal.

There is also a tendency for the quick movements to occur with the eye deviated, i.e., the quick movement does not bring the eye back to the primary position, as normally occurs, but only partially (from one- to two-thirds of the normal arc). This tend-

ency for the quick movements to occur on deviation is generally the case, but in some dogs the quick movement does bring the eye back to the primary position.

Complete hemi-decerebration and extensive injury to the thalamus do not abolish the quick component of nystagmus. This statement is based upon observations made on eight dogs that lived from two weeks to eight months following the operation. In some of these animals the lesion was produced in one operation, in others in two operations. Thirteen animals were operated in order to get this group of eight. Five of these animals died in from one to five days showing various symptoms, as continued running movements, howling and crying, rapid respiration, vomiting, subnormal temperature, deep depression and coma. These five animals did not show the quick component at any time following the operation. Three of them showed deviation, the other two no eye movements (marked deviation down and out was present) at all on rotation. In one animal the left third nerve was torn from the brain stem. Four of the eight animals kept alive had to be fed and watered daily by artificial methods, as they would neither eat nor drink voluntarily.² All of these animals died of nutritional disturbance.³ The other four dogs ate and drank voluntarily in four to ten days after the operation. The lesions were verified by autopsy and brain remnants preserved for later anatomical study.

In two animals with complete hemi-decerebration and lesion to the lateral portion of the thalamus, the remaining motor cortex including the eye-motor area and basal portion of the temporal lobe was extirpated. Both of these animals showed an increase in the postrotatory nystagmus when rotated to the side opposite the fresh lesion. This latter operation was done six

² One was nourished via gastrostomy.

³ Two of these animals during the last week of their lives, when their temperature was subnormal, passed in the stools particles of undigested food—particles of meat whose substance was not even discolored and milk no further changed than coagulated. These two animals and three others dying from extensive thalamus lesions passed liquid red-paint colored stools, which gave positive tests for blood. Autopsy of the gastro-intestinal tract revealed petechial hemorrhages and hyperemia of the gastric mucosa, the rest of the intestinal tract being normal.

to seven months following the complete hemi-decerebration. One of the animals was kept alive four weeks, the other was killed by a whelping bitch five days after the operation.

Failure has attended all attempts to produce a complete decerebrate dog. All these animals were markedly depressed and comatose and did not live longer than four days. None of these animals manifested the quick component of nystagmus. The slow component, or rotatory deviation, was present in all but

TABLE 3

Effect of complete left hemi-decerebration and extensive lesion to the thalamus on the quick component of nystagmus in the dog

DOG	NORMAL				AFTER OPERATION ¹			
	Rotatory		Postrotatory		Rotatory		Postrotatory	
	Rotated to		Rotated to		Rotated to		Rotated to	
	Right	Left	Right	Left	Right	Left	Right	Left
33	4	4	2	2	3 ²	6	22	4
31	5	5	6	6	3 ²	7	40	7
34	4	5	4	4	2 ²	7	42	9
27	4	4	5	7	0-3 ²	6	50	10
30	5	5	7	6	4 ²	8	24	5
5 ³	6	5	9	8	6 ¹	9	35	9
14	5	4	7	7	— ⁴	—	—	—

¹ Observations are taken from records two days after the operation. For interpretation of numbers see footnote to table 2.

² One to four postrotatory movements occurred. See table 2.

³ Eight months after the first operation and five months after the second, the after-nystagmus was six when rotated to the left, and sixteen when rotated to the right.

⁴ Protocol only states that quick component was present when rotated in both directions with a marked increase in the postrotatory nystagmus when rotated opposite the side of the lesion.

three. In most instances the slow component disappeared from three to twelve hours before respiration ceased.

The nearest approach to complete decerebration and destruction of the thalamus in the dog was made in dog 27 (table 3), which was a complete left hemi-decerebrate with destruction of the left lateral portion of the thalamus. This animal lived three weeks. Three days before it died its temperature became sub-

normal (32° to 35°C.) and it was very depressed, being comatose forty-eight hours previous to death. The quick component was absent during the last forty-eight hours. Autopsy revealed the thalamus to have undergone complete malacia. At the time observations were being made on this dog I was not aware of the effect of body temperature upon the quick component of nystagmus, and hence did not study the effect of raising the body temperature as was done in the case of the rabbits reported above.

A clean ablation of the occipital cortex in the dog does not alter vestibular nystagmus as judged by the results from such a procedure in three dogs.

DISCUSSION

Tozer and Sherrington ('10) have demonstrated histologically and physiologically the presence of sensory tendon nerves in the extrinsic eye muscles which pass back to the midbrain via the IIIrd, IVth, and VIth nerves. Wilson and Pike ('15) suggest that afferent impulses from these tendon nerves "set up efferent impulses in the oculomotor cells of the cerebrum, which result in a quick, jerky contraction of the internal rectus on the side of the slow deviation and of the external rectus of the opposite side, with relaxation of the antagonistic muscles," which effects a restoration of the eyes to the primary position. In other words, these latter investigators are of the opinion that the quick component is dependent upon the presence of the neopallium or upon the integrity of a cerebral reflex arc. The presence of true vestibular nystagmus in the frog, pigeon, and turtle questions this idea from the viewpoint of comparative anatomy. The persistence of true vestibular nystagmus following the removal of the cerebral hemispheres in these forms) which is a very simple matter and causes no great physiological disturbance—questions this idea physiologically. The observation that no disturbance of nystagmus follows decerebration in these forms, while in the higher forms (rabbit, cat, dog) there is a change in the number and duration of the nystagmic movements, shows that some constituent is absent in the cerebrum of the frog, turtle, and pigeon which is present in the cerebrum of the rabbit, cat, and dog.

The presence of a motor cortex in rabbit, cat, and dog is well recognized. Such an area has never been demonstrated in the cerebrum of the frog and pigeon, while it has been alleged by Johnston ('16) for the turtle, which, however, is still a mooted question. So, from the standpoint of comparative anatomy and physiology, the idea that the quick component of nystagmus is dependent upon the integrity of a cerebral reflex arc is hardly tenable.

The increase in nystagmus in animals with lesions to the eye area of the motor cortex when the slow component is directed opposite to the side of the lesion is explained by the withdrawal, as a result of the ablation, of the well-recognized heterolateral inhibitory influence which the cerebral cortex exerts over reflexes. The absence of any disturbance of nystagmus by ablation of the cerebral hemispheres in those forms that have not acquired this cerebral inhibitory function is evidence, I take it, in favor of this idea. An attempt at an explanation of the apparent diminution of the nystagmus in completely hemi-decerebrate animals when the slow component is directed to the side of the lesion will not be made in this report (tables 2 and 3).

Further, the observation that in the rabbit complete decerebration and destruction of the thalamus can be performed without abolishing the quick component shows conclusively that the quick component of vestibular nystagmus is dependent only upon the integrity of the afferent-efferent nerves of the eye muscles and their centers in the midbrain. The findings on the dog support this view.

Why depressed and comatose animals show deviation of the eye on rotation and no quick component is a question which cannot be answered directly. If the body temperature is subnormal, the quick component can be restored by raising the temperature to the normal. In other conditions of depression the quick component disappears, while the slow deviation persists, e.g., narcosis. It should also be pointed out that, although we are dealing with an entirely proprioceptive phenomenon, vestibular nystagmus apparently involves two kinds of proprioception, the quick component due to a segmental proprioceptive con-

trol, the slow component, or deviation, due to stimulation of the labyrinth, which exerts an intersegmental control (Sherrington, '06 a). Then, since some reflexes are more easily interfered with than others (Sherrington, '06 b) it is reasonable to believe that the quick component of nystagmus is a type of reflex of lower intensity, more easily interfered with and suppressed than the slow component, or deviation.

CONCLUSIONS

(Rotation was the stimulus used to produce vestibular nystagmus.)

True vestibular nystagmus is present in the frog, turtle, and pigeon. If the body temperature of the frog and turtle is below 10°C., the quick component disappears while the slow component persists.

Decerebration in the frog, turtle, and pigeon does not disturb vestibular nystagmus.

The decerebrate pigeon with extensive lesion to the thalamus manifests true vestibular nystagmus, provided its body temperature is kept normal. Otherwise, the quick component disappears and only deviation persists.

Hemi-decerebration in the rabbit, cat, and dog causes an increase in vestibular nystagmus when the slow component is directed opposite to the side of the lesion. When the slow component is directed to the side of the lesion it is not abolished, although not infrequently it is diminished.

Complete decerebration with extensive destruction of the thalamus in the rabbit does not abolish the quick component of nystagmus, provided the body temperature is kept normal. Rogers' ('18) observations of the reduction of body temperature following lesions of the thalamus have been confirmed for the rabbit.

In the dog ablation of the motor cortex in the region of the eye area with the inclusion of the lateral and basal portions of the temporal lobe causes a five- to fifteen-fold increase in the number of movements and a two- to four-fold increase in the duration of the after-nystagmus when the animal is rotated opposite to the side of the lesion. There is a slight increase in the rotatory nys-

tagmus when the animal is rotated to the side of the lesion. This marked increase is more or less temporary, but some increase is permanent, as judged from animals kept eight months.

The general conclusion is warranted that the quick component of vestibular nystagmus is not due to the integrity of a cerebral reflex arc, but is dependent upon some center below the thalamus, over which the cerebrum exercises its well-recognized inhibitory influence.

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PLATE 1

EXPLANATION OF FIGURES

1 Dog. 18. Ablation of the posterior two-thirds of the left cerebral hemisphere, including the basal portion of the temporal lobe and motor cortex. This dog showed typical increase in nystagmus when slow component was directed opposite to the side of the lesion. Quick component was present when rotated in either direction.

2 Dog 31. Complete left hemi-decerebration, including destruction of the left half of the thalamus. See table 3. Seen from injured side.

3 Dog 31. Ventral view. Both third nerves and midbrain are intact. This dog showed nystagmus when rotated in either direction with an increase as shown in table 3. *a*, Third nerves.

4 Dog 32. Complete left hemi-decerebration, including destruction of left lateral and middle portions of the thalamus and anterior left half of midbrain. Nystagmus was absent in this dog which lived only three days. *a*, Right third nerve intact; *b*, left third nerve absent with injury to midbrain.

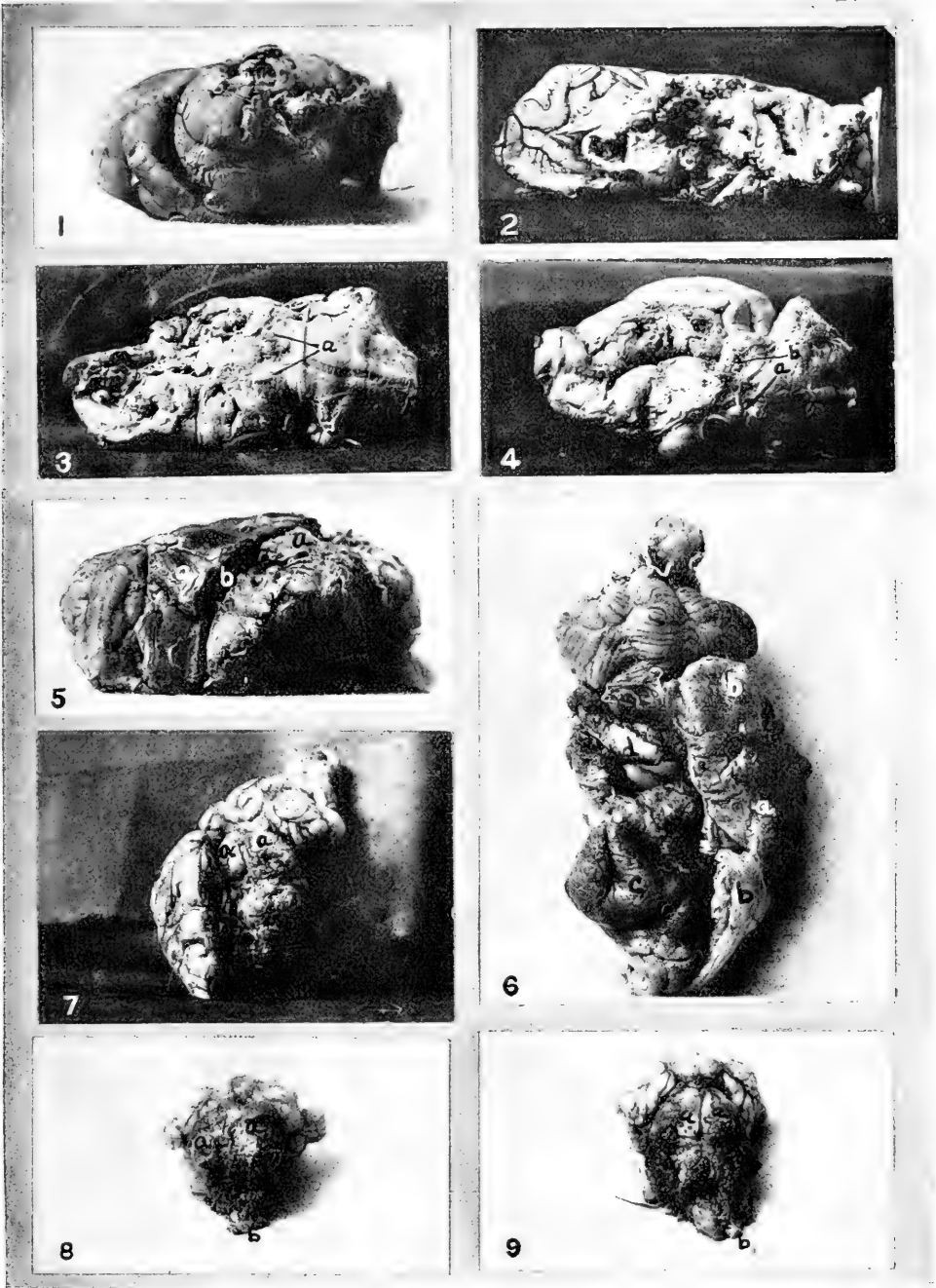
5 Dog x. Hemi-decerebration. *a*, Thickened dura; *b*, section made through fibrous tissue for examination of thalamus, which showed areas of degeneration.

6 Dog 5. Complete left hemi-decerebration with injury to lateral portion of thalamus done in two operations. Posterior two-thirds of right cerebral hemisphere removed at a third operation. There was an increase in nystagmus when the slow component was opposite to the side of the fresh lesion. The quick component was present when rotated either direction. Dog was killed three days after the third operation by another dog. See table 3. *a*, Temporal muscle drawn inwards through defect in the skull; *b*, thickened dura; *c*, hemorrhagic remaining portion of cerebrum (the only cortex present); *d*, fornix.

7 Rabbit III. Left hemi-decerebration with injury to lateral portion of the thalamus. See table 3. *a*, anterior quadrigemina.

8 Rabbit VII. Complete decerebration and destruction of the thalamus. *a*, Anterior quadrigemina; *b*, optic nerves and chiasma.

9 Rabbit VI. Complete decerebration and destruction of the lateral portions of the thalamus. *a*, anterior quadrigemina; *b*, optic nerves and chiasma.



Resumen por el autor, F. T. Rogers.
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Estudios experimentales sobre el tallo cerebral.

III. Los efectos de extensas variaciones de la temperatura del cuerpo, causadas por las lesiones del tálamo, sobre las actividades reflejas.

La extracción de los hemisferios cerebrales y el tálamo en la palomo reduce al animal a una condición poikiloterma permanente. Uno de estos animales así operado puede conservarse vivo durante un periodo de 1 a 3 meses, colocándole en una incubadora a 30°C. El comportamiento ulterior y las actividades reflejas varían con la temperatura del cuerpo. Los movimientos indecisos típicos de los animales desprovistos de cerebro aparecen cuando el animal está hambriento, si la temperatura del cuerpo es superior a 36°. Si se deja descender dicha temperatura hasta los 30° aparecen perturbaciones en el equilibrio, que se manifiestan primero por la presencia de una flexión tónica de la pata y músculos del pie. A 24° o a menor temperatura el animal no puede mantenerse en pie o volar. Los reflejos oculares, pupilares y el nistagmo desaparecen a unos 30°. Todos ellos reaparecen cuando la temperatura vuelve a ser la normal de 40°. El autor consigna observaciones fisiológicas detalladas sobre un animal después de la ablación de todas las partes del cerebro anteriores a la comisura posterior y al quiasma óptico, seguidas de un estudio microscópico de cortes seriados de las restantes partes del tallo cerebral.

Translation by José F. Nonidez
Carnegie Institution of Washington

EXPERIMENTAL STUDIES ON THE BRAIN STEM

III. THE EFFECTS ON REFLEX ACTIVITIES OF WIDE VARIATIONS IN BODY TEMPERATURE CAUSED BY LESIONS OF THE THALAMUS

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TEN FIGURES

In a previous study of decerebrate restlessness in the pigeon attention was again called to the differences in behavior of decerebrate birds according to whether or not the thalamus was traumatized in the process of decerebration. It has been long recognized by physiologists that the subsequent effects on the animal are quite different in the two cases, but a clear-cut comparative study of the two sets of conditions, followed by a careful study of the brain, is wanting. In part this has been due to want of detailed knowledge of the structure of the basal ganglia in the bird and in part to failure to recognize the rôle of secondary physiological conditions after the operation. Of the latter factors, one important factor is that of maintaining a normal body temperature, which the writer has considered in a previous report. The writer is of the opinion that there are also others, such as changes in the circulatory and digestive conditions, which also modify the physiological picture. Experiments along these lines are now under way and will be reported later.

HISTORICAL

Only a brief summary of the history of the question of the rôle of the thalamus will be given here, as it is discussed in the larger text-books of physiology, particularly those of Schaffer, Luciani, and Hermann. The more important papers are cited in the bibliography.

Vulpian in 1866 noticed that removal of the cerebral hemispheres of the carp led to continuous excessive activity of the fish, always however avoiding obstacles in its path.

Ferrier and Steiner found that removal of the hemispheres of the shark gave the same picture as followed loss of the olfactory lobes; that if the thalamus were removed, the animal lay quietly on the floor of the aquarium without movement. Bethe denied this, stating that loss of the thalamus did not abolish spontaneous movements.

Steiner, Bethe, and Loeb all agreed that damage to the mid-brain leads to motor disturbances, in the form of forced movements or circus movements, if the lesion is unilateral, in the direction of the intact half of the midbrain.

Removal of the hemispheres and thalamus of the frog abolishes spontaneous movements, according to Steiner; but not if the thalamus is left intact (Schrader).

Rolando ('09) studied the effects of decerebration in the pigeon. His observations were confined to birds which lived a few days only after operation.

Flourens ('22) continued the work and kept the birds alive for months after operation. He made no distinction between decerebration with and without thalamic involvement.

Longet ('47) was the first to attribute significance to sharp localization of brain lesions and prepared decerebrate birds with and without damage to the underlying parts.

Munk, in 1883, revived the controversy between Flourens, who maintained that the hemispheres were necessary for the various senses, and Cuvier, who thought that loss of the forebrain led merely to the loss of memory images.

Schrader, in 1889, in an elaborate monograph reported results on decerebrate pigeons in which the thalamus was carefully preserved. His primary interest was in the functions of the cerebral hemispheres and not much consideration was given to the thalamus save to make sure that it was present.

Vulpian had previously considered the activities of decerebrate animals as due automatically to stimuli "either internal or external" which incited the movements of the animals.

Munk ('90) stated that the restlessness of the decerebrate pigeon was dependent on hunger.

Exner and Onimus repeated the studies on chickens and ducks, and Kalischer more recently has tried cerebral ablations on the parrot.

Luciani has referred to the fact that the decerebrate rabbit will make spontaneous movements if the thalamus is intact.

Goltz's decerebrate dog showed continued restlessness, and in the autopsy report of Holmes it is stated that the thalamus is intact, but shrunken, due to secondary degenerations.

Bechterew and his students have attempted to get some definite experimental evidence on thalamic functions. He finds thalamic injury leading to lowered reflex excitability, forced movements which he suggests are cerebellar in origin, statis of food in the gastric organs, and a characteristic flattening of the feathers against the body instead of fluffed as in the sleeping or decerebrate bird without thalamic lesion. Furthermore, he considered that there were thalamic centers for the respiratory, digestive, circulatory, and urogenital organs.

Sachs recently tested these hypotheses by stimulation methods, and concluded that reflex effects on these organs can be obtained by thalamic stimulation, but that there are no controlling centers of these organs in the thalamus.

METHODS

Decerebration in the pigeon is a relatively simple matter. Experience, however, showed two factors particularly which should be closely watched. First, when the cranial vault is removed, care must be taken that the underlying dura mater be left intact. If this is rudely torn, mechanically the pathways for the circulation of blood through the brain stem are so interfered with as to lead to inefficient blood supply. It seems that this effect is due to damage to both the sinuses and the arteries on the lower surfaces of the brain. This may lead either to excess intracranial hemorrhage with resulting pressure complications or to deficient blood supply. Sometimes, however, it is found that the animals recover in spite of torn meninges.

The writer has been accustomed to leave a bridge of bone overlying the longitudinal sinus and then cutting through the dura, parallel to the median sulcus and to the occipital pole of the hemisphere. Hemorrhage from the large superficial artery running over the anterior surface of the hemisphere may be controlled with a cautery and the entire hemisphere removed with a blunt probe whose tip has been curved to fit around the posterior end of the hemisphere. This can be removed, the hemorrhage controlled with cotton, and a clear view of the thalamus and the third ventricle obtained. The writer has then destroyed the thalamus either by excision or by the use of a hot cautery. The latter is more satisfactory, in that it controls bleeding as well as destroying the thalamus.

In the second place, it has never been found satisfactory to leave cotton or any packing in the cranial cavity, but to allow the cavity to fill itself with blood and sewing the skin over the cavity. No attempt was made to approximate the cut edges of the dura, because of its delicacy and the fact that traction on the dura may increase the hemorrhage.

After the most careful operative work it is found that only one-fourth to one-third of the animals will live for more than a few days. These early fatalities seem to be due to circulatory disturbances. The percentage of survivals can be increased markedly if the thin medial and occipital cortex is not removed. These parts are so closely related anatomically to the large blood-vessels of the brain stem that their removal is particularly likely to be associated with excessive bleeding.

EXPERIMENTAL RESULTS

Complete removal of all forebrain substance anterior to the thalamus gives a preparation which, if the animal lives over the initial shock, conforms to the classic description. Certain features are characteristic of such an animal:

1. The bird stands quietly on one or both feet most of the time.
2. The feathers are fluffed as in the sleeping condition.

3. Digestion processes are normal if the animal is fed and watered by hand.

4. Body temperature, maintenance and regulation is nearly normal.

5. There are no disturbances of equilibrium, so that the bird can walk and fly in a normal manner.

6. The bird moves only when some stimulus, either external, as irritation of the skin, or internal, such as hunger, thirst, or defecation, disturbs it.

7. When deprived of food and water, a characteristic periodic restless walking to and fro, which ceases on feeding.

8. The loss of all typical instinctive types of actions, such as feeding, bathing, courting and mating, nesting, etc.

After three or four months there is added to this picture a restless type of walking whose causation is obscure. The bird may be restless in the day time and 'sleep' quietly at night. Furthermore, this alternation of walking in the day time and quiet at night is not merely a matter of illumination, for the blind decerebrate bird will exhibit the same type of behavior. In part it is related to hunger, but not wholly, for at this stage it may be independent of feeding or starvation. The writer has seen this type of behavior in those birds only which have survived the operation for months. This is not to be confused with a type of violent, forced, continuous walking or flying movements that sometimes appear when there has been some damage done to the thalamus or midbrain, also of unknown causation.

As pointed out by Bechterew, if the thalamus also be removed at the time of operation, the animal differs from the preceding picture.

1. The animal stands quietly without spontaneous movements.

2. The feathers lie flat against the body and not fluffed. This is so striking as to become diagnostic.

3. Digestion disturbances appear. Some of these are difficulty in swallowing, vomiting, and an apparent diarrhea or excess urination.

4. There may be indefinite or marked disturbances in maintaining body equilibrium. These may assume the most bizarre

types, such as to suggest damage to the cerebellum or to the labyrinthine mechanisms.

5. Body temperature may become subnormal. (The normal in the pigeon is from 39° to 42°C.).

6. Immediately after operation forced continuous walking or flying independently of the conditions of the digestive tract. These I have seen only during the first day or two after operation.

As recognized by Vulpian and Munk:

7. No characteristic periodic restlessness associated with hunger.

8. Loss of all instincts, as in the bird deprived of hemispheres only.

As a matter of fact, if the thalamus be removed, such animals usually die unless, 1) particular care has been taken that the operation is properly done, and, 2) great care is taken after operating to keep the animal warm and furnish some food and water by forced feeding if necessary. Furthermore, the picture of such animals after operation is quite variable, including all kinds of mixtures of the points just outlined as characteristic of thalamic injury. Examples of such behavior are as follows:

Pigeon 104

November 10. Decerebration and thalamus cauterized.

November 11. Bird walking about.

November 12. Temperature subnormal; feathers flat; bird sluggish; bird stands with difficulty with outspread feet.

November 13. Feathers slightly fluffed; bird stands quietly; pupils dilated; temperature subnormal.

November 15. Bird rejects food put in mouth; pupils constricted; reflexes sluggish.

November 16. Bird quiet; no movement. Given feed and water.

November 22. Bird stands on its feet unsteadily; pupils normal; temperature normal; reflexes sluggish.

November 23. Bird dead with crop and gizzard filled with food.

Pigeon 105

November 8. Decerebration and thalamus cauterized.

November 9. Temperature subnormal; pupils dilated; swallows water easily; bird sits on floor of cage.

November 11. Reflexes sluggish (to irritants); no spontaneous movements; pupils dilated.

November 13. Temperature subnormal; stands unsteadily on its feet; pupils dilated.

November 16. Bird seems weak; swallows water readily; unable to stand steadily; uses its tail to support itself; pupils constricted and no eye nystagmus on rotation of bird.

November 18. Bird died.

Examples of this sort could be multiplied indefinitely. Decerebration with combined thalamic lesions of one kind or another have been done on sixty birds. The details have varied considerably from bird to bird. In an attempt to analyze this variable complex, two factors stood out as at least essential. First, in each different experimental animal it is probable that there were some detailed differences in the amount of brain-stem tissue injured; and, secondly, attention was gradually drawn to the possible influence of the body temperature variations. This latter problem was then approached and a specific study made of body temperature regulation after thalamic injury. This has been reported elsewhere in detail.

With reference to the first factor, it seemed evident that it could only be controlled by a careful series of histologic preparations of the remaining brain tissue after a detailed study had been made of the behavior of the animal during life. In accordance with these principles, a bird was prepared by the usual method of decerebration with what was thought to be a thoroughly complete destruction of the thalamus. A careful record of the behavior of this animal was kept with due regard to the control of the body temperature, and after death serial sections were cut of the parts of the brain that were still intact. The writer can therefore state definitely the changes in behavior and reflex activity of this bird with wide changes in body temperature with a definite statement of how much of the brain was histologically intact at time of death.

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Pigeon 126

June 21. Bird decerebrated and thalamus cauterized with a hot probe.

June 22. 9.00 A.M. Temp., 36°C. Bird sluggish; stands with difficulty; toes are slightly arched upward so that the bird tends to stand on the claws only. (This condition is referred to as 'claw foot' in the laboratory record.) Bird put in a warm incubator kept at 32°C.

10.00 P.M. Bird is walking around; temperature of bird is 43°C.

June 23. Incubator in which bird is kept adjusted to 30°C., and bird stays in incubator until July 1.

June 26. Bird is preening itself. Is fed and watered by hand. No difficulty in feeding. Temperature of bird, 41°C.

June 28. Temperature of bird 38°C. Rotatory and post-rotatory nystagmus of eyes when bird is rotated. Equilibrium normal; feathers fluffed. Difficulties in feeding, as bird rejects much of the food put in the mouth. By use of much water, however, some is swallowed.

June 30. Bird quiet; no restlessness when starved.

July 1. Bird removed to room temperature (24°C.). Temperature of bird falls to 37°. Pupillary reactions to light present but sluggish.

Size of pupil, bright light, 3 mm. diam.

Size of pupil, dim light, 4.5 mm. diam.

Change in size of pupil with every winking movement of the eyelids.

Vomiting. Respiration, 22. Equilibrium normal. Bird perches on my finger.

July 2. 10.00 A.M. Bird has been in cool place over night. Body temperature, 33.5°. Respiration, 17. Slight tendency to claw foot. Slight disturbances in maintaining balance on a perch. Bird preens itself. Bird put in incubator at 34°.

11.00 A.M. Bird squatting on floor. Temperature of bird 36°. Preening.

Bird has been starved for forty-eight hours, but no restless walking movements occurred.

4.00 P.M. Temperature of bird has risen to 40°. Typical decerebrate restlessness; bird walking about the cage. This does not cease when bird is given excess water. Bird is picked up by hand and put down again. Walking movements stop momentarily and then resumed.

July 4. 6.00 P.M. Very hot day. Temperature of room 34°. Bird has body temperature of 44°. Bird walking around its cage all day. Given water and it becomes quiet. Feathers fluffed in normal way. Pupils widely dilated.

10.00 P.M. Temperature of bird 39.5°. Temperature of room 30°. Bird standing quietly asleep on one foot. Feathers slightly fluffed.

Turn on light in cage suddenly. Bird begins walking around. Turn off the light and bird becomes quiet. Again the light was turned on. The bird appears to wake up. Turns its head, moves a few steps, hesitates, and then begins walking around the cage. Light removed. The bird continues walking in semi-darkness and walks against the walls of the cage. Repeats this several times.

July 5. 8.00 A.M. Bird fed. Room temperature, 28°. Temperature of bird, 37°. No restlessness during the day; stands sleeping.

3.00 P.M. Sudden change in the weather. Rain and wind. Atmospheric temperature falls to 22°.

10.00 P.M. Temperature of bird has fallen to 33°. The bird stands on its feet with difficulty supported by its tail: or bird lies on floor of cage. Unable to perch on my finger.

Nystagmus of eyes, rotatory and post-rotatory barely detectible when bird is rotated. Nystagmus sometimes uncertain or it may appear after a long latent period. Nystagmus can be seen if the rate of rotation is very slow; reactions of the eyes are very slow. Slight claw foot. Head nystagmus (or compensatory movements) very distinct, although the nystagmus of the eye is very sluggish. Respiration slow and deep; 12 per minute.

The toes irritated with a needle. The head is lowered and bird preens the leg or runs its beak over the toes. This was repeated fifty times in succession. Twelve times the bird brought its beak to the exact point of irritation. Movements are rather slow and sluggish, not quick and vigorous. I use a stronger stimulus to the toes. The foot was raised.

Increase strength of stimulus. Foot jerked sharply backwards.

Still stronger irritation (painful). Bird steps backward and turns around.

This kind of stimulation applied to both feet. Seventy-five times to right foot and then the lowering of the head to the right foot ceased, although the stimulus continued.

Then repeated the stimulus on the left foot; head promptly brought to the left toes twenty-two times in succession, but not to the right when right was again stimulated.

July 6. 11.00 A.M. Temperature of bird, 31.5°. Respiration, 12, shallow. Marked claw foot. Bird supports itself by tail; unable to stand without this support; unable to perch on finger. Feathers flat. Renewed irritation of toes with a needle.

Repeated stimulation a number of times. Only once as the head lowered to the toes, and then very slowly.

Strong stimulus to toes and foot slowly lifted.

Movement sluggish.

Nystagmus: rapid rotation; no quick component; slow rotation; quick component present; both rotatory and post-rotatory.

By choosing the proper rate of rotation, it is possible to show deviation without the quick component; another rate will show both deviation and quick component.

Bird put in incubator at 30°.

July 6. 9.00 P.M. Temperature of bird 38°. Equilibrium normal; swallows water easily; removed from incubator.

July 7. 8.00 P.M. Temperature bird, 32°. No feed to-day.

9.00 P.M. Temperature bird, 32°.

Slight tendency to claw foot; bird tries to walk in a clumsy fashion; tries to coo in a low difficult gurgle.

Bird preens itself; preening ceases on striking a sudden blow to the cage.

Bird makes slow circus walking movements to the right. No inhibition of this slow labored swaying type of locomotion by giving water. Walks with outstretched head and neck in a rather comical fashion. Tendency to claw foot most marked just after the bird has been handled.

Given more water; walking continues.

Pupils widely dilated. Respiration, fourteen per minute and shallow.

Nystagmus of eye: Deviation present alone if rotated rapidly.

Slow rotation; once in twenty seconds. Quick component present.

Quick rotation; once in five seconds. Quick component absent; deviation present.

Bird unable to perch on finger.

Bird put in incubator at 30° over night.

July 8. Given feed and water. Temperature of bird, 38°.

July 9. Crop still contains feed from yesterday. No restlessness. Preening at times.

July 10. Bird taken from incubator.

July 11. 8.00 A.M. Temperature of bird is 28.5°.

Bird can barely support itself on its feet. Wings outspread. Marked claw foot.

Nystagmus of eyes absent on rotating rapidly or slowly.

Compensatory movements of head to rotation very active.

11.00 A.M. Put bird in incubator at 31°.

6.00 P.M. Temperature of bird, 38°.

Bird is standing in a normal fashion. Eye and head nystagmus present, normal. Given feed and water.

9.00 P.M. Typical decerebrate restless walking movements; crop nearly empty.

July 12. Bird in incubator. Temperature of bird, 41°. Normal restless walking movements. Given water and walking movements cease for a short time and then again begin.

July 14. Bird died about 3.00 P.M.

In emaciated condition.

Young female bird.

AUTOPSY

The skin was sunken in over the medial bridge of bone so as to leave large cavities on either side of the head. The stump of the brain stem was covered by a small blood clot.

All of the cerebral hemispheres was gone except possibly a small bit of the cortical parts bordering the longitudinal sinus. Histologically, this showed no nerve fibers or nerve cells nor in the serial sections that were made could any continuity be traced with the brain stem.

The brain stem was imbedded in celloidin and serial sections cut of the entire preparation. These were stained with iron-hematoxylin.

The medulla oblongata and cerebellum showed no alteration, judged by this method of staining.

The midbrain and optic lobes showed no alteration except for a small pocket of unstained vacuolated tissue on the left side at the anterior end. The posterior commissure and oculomotor nerves are intact and stained in a normal way.

All of the optic thalamus is gone except the hypothalamus and the posterior end of the right side of the thalamus. The anterior commissure is gone. The line of excision runs from the posterior commissure above to the anterior surface of the optic chiasma in a slightly oblique frontal plane so as to slightly damage the left optic lobe and leave part of the posterior end of the right thalamus.

DISCUSSION

The points of major interest in the preceding protocol may be summarized as follows:

1. A nearly complete removal of the thalamus destroys the temperature regulating mechanism of the pigeon so that its body temperature is determined by that of the environment.

2. As the body temperature fluctuates, the behavior and reflex activities also vary.

3. If the body temperature is kept normal, the behavior of the bird resembles that of a decerebrate bird without thalamic injury in the following respects:

- a. Periodic spontaneous walking movements occur, particularly during periods of food deprivation.

- b. The maintenance of body equilibrium remains normal.

- c. The feathers may assume a fluffed condition that resembles that of the decerebrate bird, but is never as marked as in the latter.

- d. Complicated spinal reflexes involving the coordinated movement of the beak to the toes are carried on efficiently.

4. Birds with thalamic lesion and body temperature kept normal, so far as the analysis at present stands, differ from decerebrate birds with thalamus intact in the following respects:

a. A shorter period of life after operation.

b. A tendency to stasis of food in the gastric cavities, associated very frequently with vomiting.

5. If the body temperature is allowed to fall, a gradual depression of reflex activities ensues.

a. In the preceding protocol it is seen that decerebrate restlessness (walking movements) occurred with the temperature of the bird at 43° , 40° , 39° , 38° , 41° . That it did not occur during starvation at body temperatures of 36° and 37° , with one exception and that one was at 32° . The writer is inclined to think that two types of restless walking movements are to be recognized. One associated with hunger or other visceral disturbances, and a second which is independent of reflex stimuli and is automatic in the sense that it results from changes within the brain tissue only. The nature of this automatic activity cannot be stated now, but this type of restlessness is independent of either hunger or feeding and shows itself as forced continuous movements. This type of behavior is well shown in the protocol of July 7.

b. Equilibrium apparently remains normal, so far as observation goes, until the body temperature falls to 36° or less. At 33° a characteristic tonic flexion of the toes is evident which has suggested the term 'claw foot.' At this temperature the animal is unable to carry on the fine balancing reactions involved in perching. At -20° the bird is unable to stand and lies on the floor in an uncoordinated fashion with flexed toes and outspread wings (fig. 2).

c. With a decline in body temperature the nystagmus reactions of the eye become more and more sluggish, disappearing altogether at about 30° . As the body temperature falls, the rate of rotation used to elicit the nystagmus must be made slower, otherwise it may be overlooked and the deviation alone observed. Very curiously, the compensatory movements of the head to rotation may persist at temperatures below those at which the eye

nystagmus has disappeared. (This, however, may be more apparent than real, because of the smaller amplitude of the movements of the eye as compared with those of the head.)

d. Intersegmental reflexes persisted until the body temperature fell to 33° and disappeared at 31° .

e. The typical spread of reflexes was obtained with a body temperature of 33° .

f. Inhibition of the preening reflex by mechanical vibrations of the wall of the cage was elicited at a body temperature of 32° .

In order to illustrate these changes a series of four photographs are inserted to show the influence of these temperature changes on the equilibratory mechanism. These photographs were made at two-hour intervals, from the same bird at body temperatures of 33° , 26° , 22° , and 39° .

6. Sections of the brain stem are figured in this bird which had lost the power of maintaining its body temperature. Destruction of the cerebral hemispheres and major part of the thalamus abolishes the ability to maintain and regulate a normal body temperature.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Figs. 1, 2, 3, 4 Photographs of a bird with cerebrum and thalamus removed at different body temperatures. Photographs made at two-hour intervals. Body temperature lowered and raised by putting the bird in a cooled or warm incubator.

- 1 Body temperature 33°.
- 2 Body temperature 26°.
- 3 Body temperature 22°.
- 4 Body temperature 39°.

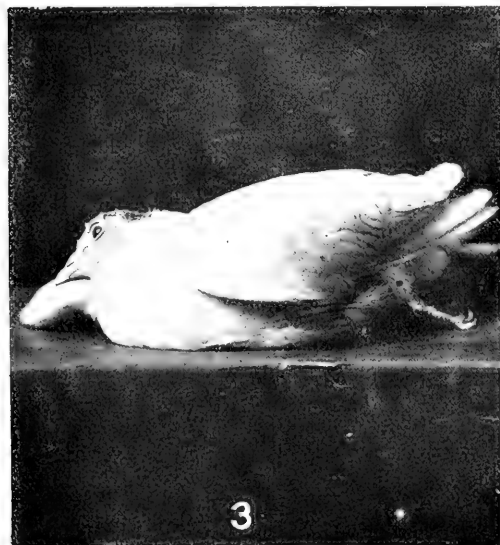


PLATE 2

EXPLANATION OF FIGURES

5 to 10 Sagittal sections taken in order passing from the right to the left sides of the brain stem of pigeon 126. Because of lack of detailed knowledge of the thalamic and mesencephalic nuclei and fiber tracts in the bird, these parts are not labeled. Camera-lucida drawings. $\times 6$.

Ce., cerebellum

Hy., hypothalamus

III., oculomotor nerve

M., midbrain

M.O., medulla oblongata

N., region of broken, unstained, apparently softened or necrotic tissue

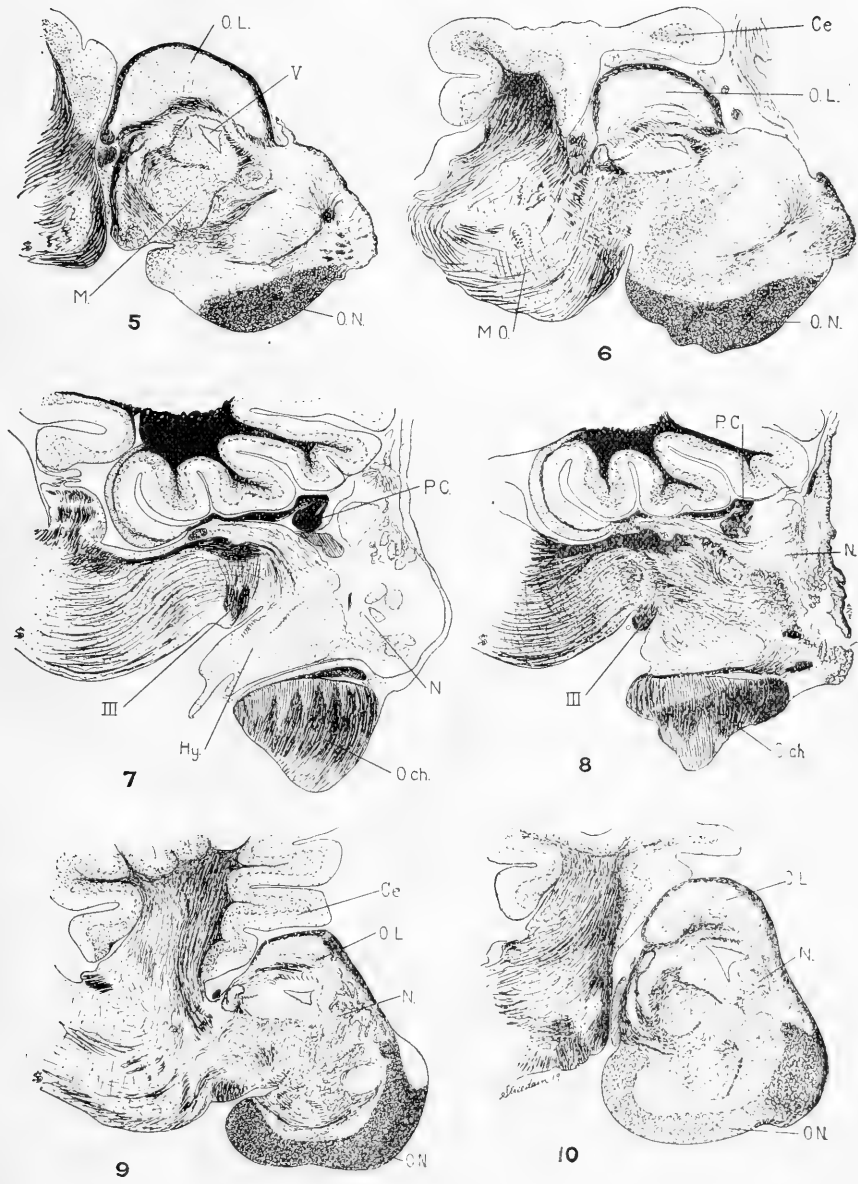
O.ch., optic chiasma

O.L., optic lobe cortex

O.N., optic nerve

P.C., posterior commissure

V., ventricle of the optic lobe



Resumen por el autor, A. T. Rasmussen.

Las mitocondrias en las células nerviosas durante la hibernación
e inanición de la marmota (*Marmota monax*).

Los marcados cambios funcionales que tienen lugar durante el comienzo y la terminación del sueño invernal en los mamíferos ofrecen una excelente oportunidad para el estudio de la relación entre los varios elementos estructurales y la actividad celular. Basándose en esta suposición el autor ha llevado a cabo una determinación cuantitativa del número de mitocondrias (condriosomas) en las principales células nerviosas de la marmota durante tres diferentes periodos: 1) Inmediatamente antes del comienzo de la hibernación; 2) durante la fase final de la hibernación, y 3) después de despertar el animal y comenzar una vida activa, en la primavera. Durante el sueño invernal o después, el animal no tomó ni alimentos ni agua. Los resultados obtenidos no indican diferencia notable en el número, tamaño, forma o reacción colorante de las mitocondrias durante los tres periodos seleccionados. El número de mitocondrias, expresado en millones por milímetro cúbico de citoplasma, en los ocho tipos de células examinadas varía entre 186 y 354. La constancia del número de mitocondrias en las células de un núcleo determinado corrobora los hallazgos de Thurlos, esto es, que hay una relación constante entre las mitocondrias y el citoplasma para cada tipo de célula nerviosa. Varias semanas de absoluta inanición a raíz de la hibernación no afectaron de un modo apreciable a las mitocondrias de las células nerviosas.

Translation by José F. Nonidez
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THE MITOCHONDRIA IN NERVE CELLS DURING HIBERNATION AND INANITION IN THE WOODCHUCK (*MARMOTA MONAX*)

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INTRODUCTION

The discovery of mitochondria (chondriosomes) in practically all living cells—except possibly in the simple blue-green algae, Cyanophyceae, and in most bacteria—and the striking similarity between those in plants and those in animals, add much interest to the question of the rôle played by these cytoplasmic granules, as is evident from the increasing number of papers appearing on the various phases of the subject. Since excellent general discussions are found in many publications, none will be attempted in this brief report. Four of the more recent papers, by E. V. Cowdry ('16 a, '18), N. H. Cowdry ('17), and by Duesberg ('19), taken together cover the subject most admirably. E. V. Cowdry's 1918 article is unusually comprehensive. It is fully evident from the literature that the function of mitochondria is a much disputed question. The widespread distribution and great similarity wherever found naturally suggest some close connection with fundamental cell processes.

Alterations in the number, size, shape, and staining reaction as a result of degrees of cell activity has been described to some extent especially in glands. But the literature on the correspondence between mitochondria and functional states in nerv-

ous tissue is extremely limited. Strongman ('17), who investigated the results of muscular fatigue in white mice, found no constant variation due to such activity, except possibly a tendency toward a clumping together of the mitochondria in the fatigued animals. This effect was especially apparent at the base of the large dendrite of Purkinje cells in the cerebellum. Luna ('13) found that shortly after cutting a large peripheral nerve trunk in the toad the mitochondria of corresponding ganglion cells lost their regular distribution, increased in size, and showed an increased affinity for the iron-haematoxylin stain. In more advanced stages of degeneration the mitochondria disappeared entirely. Busacca ('15) found that stimulation of the eye of the toad with light caused a decrease in the number of mitochondria in the pigment cells of the retina.

Earlier observations on the behavior of neurosomes—the smaller of which have been shown by E. V. Cowdry ('12) to be mitochondria—made by Levi ('96), Motta-Coco and Lombardo ('03), and by Scott ('05) indicate an increase in the number of these fuchsinophil granules during activity in nerve cells. Scott suggests, however, that such changes may be only apparent, since the entire cell is not examined, but merely the cell body, while the most important point of activity is at the nerve endings.

On the whole, it appears that mitochondria of nerve cells are comparatively resistant to conditions associated with normal processes.

In many pathological tissues (for literature see McCann, '18, and E. V. Cowdry, '18) mitochondria often show marked alterations even in early stages, and yet, according to McCann ('18), in experimental poliomyelitis the mitochondria persist in normal number in proportion to the cytoplasm in cells not only where the Nissl substance has disappeared, but also in the latest stages of neurophagocytosis.

Having seen nothing on record concerning mitochondria in nerve cells during hibernation, it seemed desirable to investigate the influence of profound dormancy in mammals, such as is characteristic of hibernating marmots, notwithstanding the fact that changes during lethargy in the Nissl granules, which are consid-

ered very labile, are doubtful (Rasmussen and Myers, '16). It was hoped that the enormous reduction in nearly all, if not all, vital processes of such animals during winter-sleep would add one more link to the chain of evidence for or against some of the theories concerning the connection between mitochondria and functional activity. Since alterations in the nervous system are important factors in all the more thoroughgoing hypotheses concerning the cause of hibernation (Rasmussen, '16 a), the facts discovered would be of interest also in connection with the mechanism involved in the production of dormancy.

MATERIALS AND METHODS

In this investigation there are involved fifteen adult woodchucks of the lot used in connection with a determination of blood volume as already reported (Rasmussen and Rasmussen, '17). The conditions under which the animals hibernated is there described. Five animals (two females and three males) were sacrificed between December 1st and December 3rd, just before the onset of hibernation, while still active and under full feed. Five others (two females and three males) were killed while dormant during the last few weeks of hibernation (between February 26th and March 18th). The remaining five (two females and three males) were bled between April 3rd and April 18th while active and after having been awake from four days to three weeks, but without having available any food or water either during or after winter-sleep.

After the blood was washed out by gradual perfusion with oxygenated Locke's fluid, warmed or cooled to the body temperature, according to the technique employed by Dreyer and Ray ('11), the vessels were flushed out with physiological salt solution and then Regaud's fixer (one part of commercial formalin neutralized with magnesium carbonate and four parts of a 3 per cent aq. sol. potassium bichromate) was allowed to perfuse the entire animal for an hour. In order to see if the length of the perfusion with Locke's solution had any noticeable effect on the mitochondria, the last animal of each of the three groups was perfused only long enough to cause a return of a colorless fluid from

the veins before the fixer was injected, as is done regularly by Cowdry. These three control animals are at the bottom of each group in the accompanying table of results. Further treatment was done according to the outline given by E. V. Cowdry ('16 b). Sections were cut 2μ thick except in the case of the spinal cord where the sections were cut 3μ . Such thin sections were necessary to facilitate and make more accurate the counting of mitochondria.

At least four consecutive sections from a given block were mounted on each of ten or more slides and stained with a 20 per cent solution of acid fuchsin in aniline water and differentiated with a 1 per cent aq. sol. of methylgreen after various degrees of dechromation. Several slides were made under what appeared to be the optimum conditions so as to insure plenty of well-differentiated sections. By having four or more consecutive sections mounted on each slide it is possible to rule out any variation in thickness which might escape general inspection by counting the mitochondria in an equal number of cells from each of the four (or a larger even number) consecutive sections and using the average. As a matter of fact, only in a few cases was there any trouble in getting uniform cutting, despite the extreme hardness resulting from long fixation and the high melting point (60° to $62^{\circ}\text{C}.$) of the paraffin. A difference in thickness in sections cut as thin as 2μ is readily noticed by the much greater tendency of the thinner ones to wrinkle, so that only such regions of the ribbon as showed uniformity in cutting were used.

In selecting the levels for study important nuclei and ganglia in which the cells have a more or less well-known function were selected, as is seen in the accompanying table of results and related data. In the case of the spinal cord the sixth cervical segment was used for somatic motor cells of the ventral cornu and the seventh thoracic segment for visceral motor cells of the lateral cornu. The seventh thoracic and sixth cervical spinal ganglia were used for dorsal root ganglion cells. In the cerebellum the cortex from the inferior portion of the vermis was utilized. No comment is necessary on the other regions selected. It is believed that sufficiently varied types of cells have been chosen to

be a fair index to the mitochondrial behavior in the nervous system under the conditions of hibernation and to rule out the objections frequently made to conclusions drawn from alterations detected in only a single type of cells (usually the Purkinje cells of the cerebellum) which often are apparently only remotely related to the physiological process under investigation.

In determining the number of mitochondria a Whipple eyepiece micrometer disc (Bausch & Lomb), on which squares of various sizes are ruled, was used. In general the medium-sized square ($\frac{1}{100}$ of the entire ruled field) was used as the unit. This area with the oil-immersion objective, ocular, tube length, etc., used and which were, of course, kept constant throughout the determinations, gave a field of $\frac{1}{13.611}$ sq. mm. In the case of sections 2μ thick the cubic volume represented by each square is $\frac{1}{6,805,500}$ cu. mm. In order to reduce all figures to the number of mitochondria in million per cu. mm. of cytoplasm the number of mitochondria found in the above small volume was multiplied by the factor 6.8. The corresponding factor for sections 3μ thick is 4.537. All figures given, therefore, indicate millions per cu. mm. of cytoplasm. For each type of cell in each animal the average of at least twenty fields from twenty different cells is given. Since there are five animals in each stage, the figure for each stage as a whole is the average of 100 cells.

As a preliminary study, the total number of mitochondria in such sections of cells as contained the nucleolus were counted. On account of the irregularity in shape and the great personal factor necessarily involved in deciding on what shall be considered the limits of the cell body, these figures are not given. They showed, however, exactly the same results as obtained by the quantitative determinations. Obviously, the results stated in terms of the number in a known volume of cytoplasm are the only facts capable of comparison with the work of other investigators. Due to the difficulty experienced, under the best optical conditions available, in determining the number of mitochondria when too closely packed, as they are occasionally in small clumps, the figures are necessarily only approximate; but they are believed to be sufficiently accurate for comparative purposes.

RESULTS

A description of the mitochondria of the nerve cells in this particular animal is unnecessary since they are essentially as described in other vertebrates by E. V. Cowdry ('12, '14), Busacca ('13), Nicholson ('16), and others. Suffice it to say that in the central nervous system they are usually granular near the nucleus and tend to become short rods more peripherally and long filamentous in the base of the processes and out in the dendrites and axon proper. In the spinal ganglion they are glandular or very short rods rather uniformly distributed throughout the cell body except in places where there is an accumulation of lipid. In such regions the mitochondria tend to be excluded. This reciprocal relation between lipid and mitochondria in spinal ganglion cells has been noted especially by Cowdry ('14) in a number of species of vertebrates. In no case, however, was there much lipid, and in spinal ganglia, where most often encountered, only two or three cells in a section through an entire ganglion would contain any obvious lipid by the method employed. But since there are innumerable gradations between granules and filaments, it was not practicable to determine any variation in the different types of mitochondria upon any quantitative basis. General examination, however, indicated no variation in shape or size characteristic of either particular individuals or of any of the three stages.

Many of what appeared to be rods are undoubtedly merely rows of two or more granules so closely packed that it is not possible to distinguish the separate components with the available apparatus. In imperfectly fixed tissue mitochondria are frequently clumped into larger masses which are clearly not representative of the normal condition. To what extent this occurs in the case of the best fixation is difficult to say. The long filaments in the axon and dendrites and in the cell body at the base of these processes are undoubtedly continuous filaments, since they are never seen as rows of granules and are always elongated continuous bodies even in the most superficial fibers in a block and next to large vessels where the fixation has been instantaneous while the tissue was still warm. Too much reliance cannot be

placed on the form of mitochondria, at least within certain wide limits and especially in regard to length, for in living cells, as was observed by the Lewises ('15), elongated ones broke up into granules and granules fused into rods and they seemed to readily bend and assume various shapes as they moved about in the cytoplasm. E. V. Cowdry ('18) has pointed out that the form of mitochondria is not correlated with protoplasmic activity or quiescence.

The long perfusion with oxygenated Locke's solution (the maximum total length being one hour) at body temperature produced no obvious effect on the morphology or distribution of the mitochondria in nerve cells. Autolytic changes after death being comparatively slow in nervous tissue, no change should be expected. In fact, E. V. Cowdry ('18, p. 138) makes the statement that it is not even necessary to fix nervous tissue while still warm; "six or eight hours after death is often soon enough." It was, however, necessary to rule out this variable.

In regard to the number of mitochondria, the accompanying table gives the results in a compact form. It is clearly evident that the number of mitochondria, as already noted by Thurlow ('17), in a unit of cytoplasm in the cells of a given nucleus is comparatively uniform. The individual variations between neighboring cells of the same type was, of course, much greater than the averages tabulated. This is due, to a large extent, to the necessity of using such a small surface area—one that will fit in between the nucleus and the periphery of the cell. If the Nissl granules are large the mitochondria are less uniformly distributed in such a small square because in general the mitochondria lie between the masses of tigroid substance. The results to be of value must be based upon a sufficiently large number of fields to eliminate this irregularity of distribution. To do this in connection with the motor cells of the ventral horn it was necessary to count the mitochondria in at least twice as many unit areas as was used in general.

The only other attempt at quantitative determination of mitochondria is that by Thurlow ('17). This was done on the nuclei of the cranial nerves of the white mouse by utilizing sections 4μ

DATE	ANIMAL			NUMBER OF MITOCHONDRIA IN MILLIONS PER CUBIC MILLIMETER OF CYTOPLASM							
	Number	Sex	Rectal temperature	Spinal ganglion cells	Motor cells ventral horn	Motor cells lateral horn	Cell of Nuc. Grac. Nuc. Cune.	Purkinje cells cerebell.	Cells of superior collic.	Betz cells motor cortex	Mitral cells olf. bulb.
Before hibernation. Active and fed											
December 1...	1-SIV	♂	35	300	185	252	253	360	229	326	261
December 2...	2-SIV	♀	36	305	174	260	266	349	335	300	260
December 2...	3-SIV	♂	36	293	181	258	254	362	246	307	246
December 3...	4-SIV	♀	36	309	195	255	261	354	244	295	259
December 3...	5-SIV	♂	35	287	178	259	242	351	236	297	262
Average				302	183	257	255	355	238	305	258
During hibernation. Dormant. Not fed since early in December											
February 26...	51-SIII	♀	7	298	180	256	254	251	240	300	265
March 3.....	6-SIV	♀	15	295	198	250	240	344	249	296	255
March 4.....	7-SIV	♂	16	310	189	258	258	359	232	305	262
March 17.....	8-SIV	♂	14	286	187	246	255	341	236	303	259
March 18.....	9-SIV	♂	12	293	194	266	249	355	243	302	265
Average				296	190	255	251	350	240	301	261
After hibernation. Awake. Active. Not fed since early in December											
April 3.....	56-SIII	♂	34	291	187	245	243	358	244	292	249
April 14.....	10-SIV	♂	34	312	177	267	260	348	220	290	266
April 14.....	11-SIV	♀	34	300	188	249	248	363	237	310	255
April 15.....	13-SIV	♀	36	312	186	260	258	355	240	299	258
April 18.....	15-SIV	♂	37	283	194	251	239	359	238	287	253
Average				300	186	254	250	357	236	296	256
Average of all animals				299	186	255	252	354	238	301	258

thick. Her results showed a variation in the number of mitochondria per cu. mm. of cytoplasm from 178 million (in the dorsal motor nucleus of the vagus) to 284 million (in the mesencephalic nucleus of the trigeminus). The final averages of the fifteen woodchucks will be seen to vary between somewhat higher limits or 186 million (in the motor cells of the ventral horn of the spinal

cord) and 354 million (in the Purkinje cells of the cerebellum). The levels selected for this investigation and the small pieces of tissue taken from these levels did not include the nuclei examined by Thurlow, so that specific comparisons can not rigidly be made. The magnitudes are seen, however, to be of the same order, except that the upper limit is considerably higher. The number of mitochondria in the large motor cells in the nucleus of the hypoglossus of the white rat was among the lowest determinations (187 million) and agrees strikingly with the number here reported in the large motor cells of the spinal cord in the woodchuck, which is also the lowest determination (186 million). As was found by Thurlow, sensory cells are not distinguishable as a class from motor cells upon the basis of the number of mitochondria.

As has been observed before, particularly by E. V. Cowdry, now and then an individual cell will contain many more or, less frequently, distinctly fewer mitochondria than the neighboring cells of the same type, which, being found in the same region of the same section, must have been through exactly the same technique. This possibly indicates that individual cells may be in quite a different condition from the vast majority. Such a situation has been assumed to explain the classical Golgi technique when, as frequently happens, only here and there a cell is picked out and hundreds of surrounding cells are left unstained. In the spinal ganglion the few cells which contained an unusually large number of mitochondria were usually of the smallest type.

This more or less specific mitochondria-cytoplasmic ratio is another argument against the view maintained by Portier (see discussion in *Compt. Rend. Soc. Biol.*, 1919, T. 82, pp. 244, 309, 337) to the effect that mitochondria are organisms living in symbiosis in larger cells.

The relationship of the mitochondria to the Nissl bodies as it appears in this investigation does not support the idea that the tigroid substance is normally more or less diffused throughout the cytoplasm and that the appearance of rather definite masses is an artifact produced by the reagents. Were this contention correct one would expect more of the mitochondria to be embedded in these precipitation products.

It further appears certain that there is no appreciable modification in the number of mitochondria as a result of the alterations attending hibernation, awakening, and subsequent inanition, thus testifying to the stability of these bodies under greatly modified functional conditions.

In the first place, during winter-sleep there is a great reduction in the metabolic processes. From the excellent summary of the literature on respiratory exchange during hibernation by Krogh ('16), it would appear that in mammals with a normal body temperature of about 36°C. when awake and whose body temperature approaches 10°C. or less during hibernation, the oxygen consumption falls to one-twentieth or less of the amount used before dormancy occurred. The CO₂ eliminated decreases relatively much more. This great reduction in oxidation processes in the body does not apparently affect the mitochondrial content of nerve cells, although in all probability the nervous system shares at least to some extent in the reduced oxygen consumption. There is, then, from this source no evidence in favor of the theory that mitochondria are associated with oxidation processes. The possibility exists, however, that they may be involved in such processes without showing any morphological or numerical changes with degrees of activity. N. H. Cowdry ('18) found in myxomycetes that the mitochondria were found in all stages of the organism, even in fully formed spores with a thick horny capsule and supposedly in a state where the physiological processes are nearly at a standstill.

What other tissues in the woodchuck will show remains to be determined. The glands are now under investigation.

During hibernation the absorbing power of the blood for CO₂ decreases and there is a distinct increase in the amount of CO₂ actually found in the blood (Rasmussen, '16 b). These changes as found in the venous blood reflect an increase in the H-ion concentration of the tissues. This tendency toward acidosis does not seem to have any effect on the mitochondria in nerve cells, although there must be readjustments in the nervous system to this altered condition of its blood supply.

Next we may mention the relation of mitochondria in nerve cells to the transition of a mammal from the warm-blooded (homoiothermal) type with a body temperature of about 36°C . to what is in many respects the cold-blooded (poikilothermal) type with a temperature only slightly higher than that of the surroundings, and which reached as low as 7°C . in one animal here involved. This striking alteration seems to have had no effect.

Finally, attention is drawn to the fact that during dormancy and for as long as three weeks after waking up, i.e., until the last animal was killed, no food or water was available. This inanition during hibernation as well as after becoming active did not apparently affect the mitochondria of nerve cells, although during the three months of winter-sleep the body weight decreases about one-fourth while the animals allowed to live several weeks after waking up lost fully one-third.

SUMMARY

1. Profound dormancy such as is seen in a fully hibernating marmot with a rectal temperature as low as 7°C . does not affect noticeably in any way the mitochondria of the central nervous system or of the spinal ganglion.

2. Complete inanition for three months during winter-sleep and for three weeks after waking up does not modify the morphology, number, or distribution of mitochondria in nerve cells.

3. Perfusion with oxygenated Locke's solution at body temperature for a period as long as one hour does not modify the mitochondrial content of nerve cells beyond what is produced by a more rapid flushing out of the vessels for a duration of only fifteen minutes.

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ERRATUM

THE JOURNAL OF COMPARATIVE NEUROLOGY, volume 31, number 1, October, 1919, page 28, line 28, "At -20° the bird is unable to stand" etc., should read, At 20° the bird is unable to stand. This slip should be pasted on page 29 when the volume is bound.

Resumen por el autor, C. U. Ariëns Kappers,
Amsterdam.

El caracter logético del crecimiento.

Los diferentes factores que toman parte en nuestra experiencia consciente o en la construcción de nuestras concepciones mentales, tales como la asociación, memoria, atención y la ley de Weber, son reconocimientos conscientes de las propiedades generales de la vida, los cuales pueden demostrarse úgualmente en el desarrollo inconsciente del cuerpo. En el acto de pensar se experimenta directamente su influencia o se reconoce por introspección (en las percepciones, por los resultados de algunos estímulos); en el crecimiento del cuerpo aparecen sin embargo, "de facto." La vida mental y el desarrollo corpóreo también están relacionados entre sí bajo este aspecto: en que la unidad de nuestro ser en ambos precede a las influencias externas, cuyas influencias en ambos casos actúan expotencializando la "multiplicidad del yo"¹ (ego) primaria. En la vida mental las asociaciones de esta multiplicidad primaria producen una multiplicidad secundaria durante la vida personal, la cual es mucho menos coherente que la primaria. Por consiguiente la consciencia del factor de la atención solamente puede abarcar unidades, bien sea en un sentido espacial, tal como sucede con los objetos concretos, o bien sean unidades abstractas, unidades en un sentido espiritual, tal como sucede con las leyes. La multiplicidad del ego en su ambiente está, sin embargo, fuera del propósito de la consideración científica atenta; su presencia se reconoce y experimenta. La atención toma una parte en ella pero no la revisa.

¹ "many-in-oneness."

THE LOGETIC CHARACTER OF GROWTH¹

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TWO FIGURES

That growth is wonderfully logical in its results, as a rule, has often struck biological students. This has led me to raise the questions: Is there perhaps a connection between what we call logical reasoning and the logic of growth? May the rules of logical reasoning be analogous to those in the logic of growth?

This question has been raised before² in the following form: May introspection be considered not only as a psychological method (nobody will doubt that), but also as a biological one? In other words: Have peculiarities which we experience in our psychic life a general biological significance, and if so, to what extent?

The answer to this question will not always be the same, and in general it may be said that great circumspection is necessary here, and that, though there may be common points of agreement, the characteristic nature of the rational functions, of the instinctive actions, and of the somatic development should not be lost sight of.

Hence, when, in 1870, Hering delivered his lecture in Vienna, "On Memory as a General Function of Organized Matter," he rightly began with the following very true words:

When the naturalist leaves the workshop of his limited special researches, and ventures on an excursion into the domain of philosophical speculations, where he hopes to find the solution of those great problems for the sake of which he devotes his days to solving the small

¹ Read before the Sixteenth Dutch Congress for Natural and Medical Sciences. The Hague.

² See, for instance, C. J. Herrick, Introspection as a biological method, *Jour. Philosophy, Psychology and Scientific Methods*, vol. 12, 1915.

ones, he is accompanied by the secret fears of those whom he leaves behind at the work-table of special research, and he is received with justifiable distrust by those whom he salutes as the denizens of the empire of speculation. . . . Thus he is in danger of losing with the former and of not gaining with the latter.

These words are very true, but still this danger did not make him refrain from expressing his thoughts on a subject that we all as biologists both love and fear—natural philosophy in the widest sense of the term.

That this same danger threatens me, who not only consider memory, but also association and attention (concentration) as general functions of organized matter, is clear. It was therefore, not without some hesitation, that I sent this paper to the editor of *The Journal of Comparative Neurology*. Its title sounds a little bold in the ears of most biologists.

I also thought it better at first to change 'logetic' in its title into 'logical.' Since, however, we are accustomed to consider logic, reason, as something that is peculiar to conscious thinking, and there will be question here of a general principle of life, which, with other faculties but according to similar laws, also operates outside conscious thinking, I have preferred to use the word 'logetic' to indicate a broader idea of 'logos', formerly used to give expression to something that is more than that small part of reason of which we become conscious in our 'logical' thinking.

I do not want to be misunderstood. I do not mean to say that logical 'thinking' accompanies the somatic development, nor that a tissue differentiation of the same form that obtains in the soma accompanies the building up of our spiritual life. No spiritualization of the somatic, therefore, nor a materialization of the spiritual. I only want to point out that one and the same principle of life, which Aristotle called 'psyche',³ with other faculties, but ruling with similar conformities, is peculiar to both, and leads in both to results which are different in effect, but which agree in

³ So this is a psyche in a much wider sense than it has been used in the word 'psychology.' Cf. Hammond, *Aristotle's Psychology. A Treatise on the Principle of Life. De Anima*, book 1, chapter 5, Alinea 31: "parts of the soul are all found in every one of these bodily divisions and they are of like with each other and with the entire soul."

being adapted to the influences of the environment in a rational manner.

ASSOCIATION (CORRELATION) IN SOMATIC AND MENTAL DEVELOPMENT

One of the striking factors in the construction of mental conceptions is the preponderant rôle which the simultaneity or direct successivity of stimuli plays in them. This was realized by Aristotle and has always been confirmed. It has also been found that in all forms of association it is the simultaneity of stimuli or residua of stimuli that act the chief part.

Similarly comparative anatomy of the brain shows that the neurons in the central nervous system⁴ always effect connections between two areas, which (even before the neuron joins them) stand to each other in a stimulative correlation, that is to say, which are often simultaneously or successively in a condition of irritation.

This stimulative correlation—in keeping with the chief law of neurobiotaxis—precedes the anatomic relation (the formation of the neuron which will join them) and this neuron junction is the result of it. In other words, simultaneous irritations, which repeatedly penetrate into the nervous system in different places, cause in this nervous system a neuronic association⁵ or associative integration between the centers where they arrive.

This law is for the material development of the nervous system the same as what we have known for centuries as the law of association in our conscious conceptions (though it was discovered independently of it, without any psychological afterthought).

Now, if we consider what we observe in the development of a germ-cell into an organism, we find there, too, simultaneously two poles which are conspicuous in the division of the germ cell,

⁴ That this also obtains for the peripheral nervous system has been lately proved by Bok in a very ingenious article. Vide *Psychiatrische en Neurologische Bladen*, 1917, no. 4, "The reflex circle."

⁵ What the physicochemical processes are that attend the formation⁷ of this neuronic linking, I cannot discuss here; I beg to refer the reader to *The Journal of Comparative Neurology*, vol. 27, 1916.

and which are mostly indicated by the two centrosomes. So here, too, there are two (sometimes more) simultaneous centers of influence, which play an essential part in the accomplishment of the process.

Whereas, however, the simultaneous action of influences causes a 'linking,' an *association* in the construction of mental life and also of the nervous system, there appears a *differentiation* in the other case (in somatic development), a differentiation which, however, remains a unit, an individual; in other words, the 'linking' of the parts which is a consequence of the process in mental life, is present at the starting-point in somatic development and persists.

Speaking properly, however, it may be said, that here, too, the 'linking' of the results of those influences does not arise till the differentiation has been accomplished, because, when the germ-cell was still one cell, the influences, which bring about the differentiation, had not yet acted, and so (apart from engrammatic factors) the results of those simultaneous influences, too, could not as yet have been linked.

So in both processes there are simultaneous influences, from which originates a formative process, in both a linking of those influences; in the cerebral linking, however, an integrated association of them and in the development of the germ-cell a differentiated association. In both cases, however, there arises a construction, which is a product of correlated influences of the surroundings.

Let us consider in this light the influence of the medium on the differentiation of the cells. In order to explain how the division and multiplication of the cells is at the same time attended with a qualitative differentiation of the daughter-cells, it is supposed on good experimental grounds that the two sides of the mother cell, owing to their different situation—owing to the fact that they are exposed to different influences—do not undergo the same differentiation.⁶ That from the same blastomeres entirely

⁶ Wilson (also Driesch and Hertwig): "The relative position of the blastomere in the whole determines in general what develops from it; if its position be changed, it gives rise to something different; its prospective value is a function of its position." (The cell in development and inheritance.)

different differentiations may result by only changing their position with regard to their environment has been shown by Morgan by his experiments on the development of frogs' eggs.

It seems probable to me that these different influences operate upon the centrosomes and by means of the centrosomes on the rest of the cell and the nucleus. It seems probable also that in this way each centrosome introduces different somatic properties into the plasm of the daughter cells, so that by means of the centrosomes not only an increase of the cells is effected, but also an adequate organoplastic differentiation.

In this connection it seems to me of great importance to inquire whether in malignant new growths where the adequate organoplastic formation of cells is absent the centrosome has undergone a change. The fact that the centrosome, or the substance from which it is derived, is very sensitive to external influences makes it easy to believe that the centrosome can become ill under the influence of inadequate processes and this illness of the centrosome might include the failure of organoplastic development.

My opinion about the centrosome as an intermediary of extracellular influences seems to be confirmed by the relation of the centrosomes in sense-cells, where they are always found in that part of the cell that is turned to the external side which receives the influence.

In the larval retina they are found (Fürst) as real centrosomes in the receptive part of the neuro-epithelium. In the adult rods and cones they are found in the 'Aussenglied' (Kolmer), while in the olfactory cells, auditory cells (Held), and cells of the saccus vasculosus (Dammerman) they are attached (eventually as diplosomes) to the hairs which project into the surroundings (fig. 1). This clearly demonstrates that the centrosomes have to do with external influences.

The same is seen in nerve-cells. In the embryonic nerve-cell, the neuroblast, the centrosome generally lies near the pole where the first offshoot of the nerve-cell, the axis cylinder arises (Held). The position of the fibrillogenetic zone, so says Held, always coincides with the position of the centrosome.

Since we have to accept that the fibrillogenetic zone is the part that is first subject to formative (stimulative) influences, it follows that in the embryonic cells the centrosome coincides with the primitive stimulative center. When later the dendrites have arisen, they form the chief receptive apparatus for stimuli, and it is not strange to see the centrosome of the adult cell near the dendrites.

In the ganglion-cells of the retina O. v.d. Stricht found the centrosome in the dendritic part of the cells and so did N. v.d. Stricht in young spinal ganglion cells still in the bipolar stage. (In adult monopolar ganglion cells they seem to lie often near

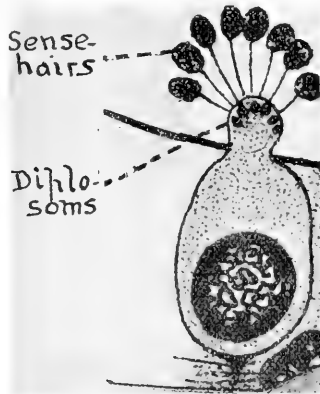


Fig. 1 Sense cell of the saccus vasculosus of *Pleuronectes limanda*. After Dammerman.

the monopolar offshoot). Hatai also found them in the cells of the spinal cord and in the Purkinje cells nearly always in that part of the pericaryon which is directed toward the dendrites (the same seems to prevail in the drawings published by del Rio Hortenga and others).

This position of the centrosome near the place which receives the influences from the surroundings reminds us of the structure of the spermatozoid where it is attached to the flagellum, and the same applies to ciliated epithelium.

All these facts lead us to believe that the centrosome may receive influences from the cell environment and support the supposition that the centrosome also during division may be the center by which influences from the environs of the cell are received and activated.

In metazoa where the cells are heaped together this cannot be proved histologically, but the relations found in several Protozoa seem to support this opinion. The centrosome in a 'dividing' protozoon can have a material relation with the outer world and can readily suffer an influence from the outer world during the partition.

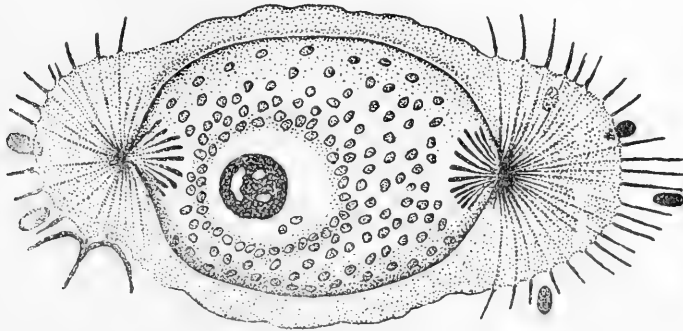


Fig. 2 Mitosis in *Stypocaulon*. The polar radiation is connected with extracellular offshoots which are subject to external influences. After Swingle.

This is demonstrated by the *Lophomonadidae*⁷ and proved also by cases like *Stypocaulon* (fig. 2), where the centrosomes of a dividing cell are attached to protoplasmic fibers which project into the environment.

As to the manner in which a cell of the body responds to the influence transmitted by the centrosome, nothing can be said as yet. It would seem, however, that this response is a striving of the body after equilibrium.

Probably the somatic response will be such that the equilibrium disturbed by the irritation from without is restored; that is, it is

⁷ Cf. Hartmann's *Protistenstudien*, Fischer, Jena, and Doflein's *Protozoen-Kunde*.

a reciprocal action, based on a striving after equilibrium, a contrary differentiation or manifestation of energy which can react toward a defect with a proliferation, toward pressure with increased hardness, toward light with pigment, toward toxin with antitoxin.⁸

Moreover, the organism whose reciprocating energy has thus been evoked remains throughout a unit materially and functionally, viz., it shows in all its parts an associated correlation, and the developed organism manifests itself as one correlated system whose harmony is astonishingly reasonable in a logetic sense.

One need only think of the relation between lens, retina, and pigment in the eye, the mutual development of which far exceeds in logical, or rather logetic, relation the mental possibilities of our conscious logical intellect.

Thus, there is in our somatic development a logetically correlated relation which has its origin in the same cause as the mental associations, viz., in different but simultaneously operating, i.e., correlated, influences.

In this development of form the 'function' is inherent of which the 'logetical' relation with the surrounding world and with the rest of the body is not less evident, and operates with the exactness of mathematical reasoning; witness the different ways in which accommodation of vision is effected in the animal series.

It appears, therefore, that the *associative differentiation of the body* is in its result a different thing from the *associative linking in the nervous system*, but that both of them find their origin in *correlated stimuli*.

Both the neuronie linking and the building up of our conscious mental life, on the one hand, and bodily differentiation, on the other, are reasonable correlations, originating in correlated irritations, two different forms of logetic realization.

Besides these, there are in both processes other common factors, which again manifest themselves in each in a different way,

⁸ The manner in which nature forms its somatic images differs from that in which its mental images are conceived in that the latter are not contrary to the influence in the sense just described.

but which may perhaps be shown to be identical, viz., memory, Weber's law, and attention.

MEMORY AND THE 'EGO' IN SOMATIC AND MENTAL DEVELOPMENT

On memory I shall not dwell long. Better qualified men (Hering, Laycock, Butler, Semon) have pointed out the part which engrammata may play in all organized matter and the importance of their reproduction, their 'ekphorie,' as Semon calls it.

That authors have occasionally gone too far in the 'engram theory' and in what respects, I will not discuss for the present. Here I will only point out a special part which the engrams play in our conscious or subconscious mental life and what analogy they have with our bodily life.

In this connection I must point, in the first place, to an apparently very great contrast between the construction of our mental life and the differentiation of our body. For, whereas the physical development begins as a real unit—the germ cell—which contains all potentialities, and this unit is sustained in the developed individual, the conscious integration of the observations and conceptions is, on the contrary, very incoherent in the beginning, and besides confined to very few data, as we know from our own spiritual development.

The phylogenetic development of the brain, too, would seem to show that the integration of impressions advances but gradually. In fishes the forebrain, midbrain, oblongata, and spinal cord function to a large extent autonomously. Only in the higher animals, and especially in man, is there a greater linking or integration by the association of everything, or at least of a great deal, in the cortex cerebri.

In the mental integration (as well in the phylogenetic integration of brain functions) the coherent unit seems to come only as a final result, indeed, is only very incompletely reached in this final result as the many 'gaps' in our knowledge prove.

Concerning ourselves, the contents of our spiritual life, however, are not built up merely by secondary linking of observations, for as we know by experience, every separate observation and stimulation falls into the primitive but in a certain way com-

plete ego, which seems to be present in the nerve-cell as a derivative of the germ-cell; and from this it follows, not that 'the light is to be seen,' but that 'I' (i.e., the primitive many-in-oneness of potentialities) 'see this light' (cf. also Hughlings Jackson, Pick, and others).

All perceptions⁹ and correlations always lie in this ego, which may represent the primitive many-in-oneness of mental life. In these perceptions, however, the ego stands in the background of one's consciousness. Indeed, it is not seldom in the first instance made active by a perception, which it precedes, however, in potentiality.

It seems now probable to me that here, too, this 'ego,' i.e., the direct experience of myself, the primitive unit, is bound to all nerve-cells, and that owing to this the consciousness of self (not the secondarily formed conception of myself) can remain, notwithstanding large destructions by illness, which it would not be possible to explain in an exclusively secondary linking of the different neurons in a very imperfect secondary 'ego.'

The secondarily integrated conscious image is very incomplete of its kind, and human ingenuity would require much more than a lifetime of observations and experiences to build up, in secondary integrations, all that which works as spiritually active factors in the individual ego.

The 'egoity' awaked by influences from without includes, however, undoubtedly much more than lifetime experience and begins with a completion (be it un- or subconscious) which bears a perhaps infinite series of engrams and peculiarities, which in our subconscience are joined 'intuitively' (in an 'entelethic'¹⁰ way).

⁹ These perceptions preserve a certain separation because the interval also represents a situation of the ego.

¹⁰ The word 'entelechia,' first used by Aristotle, comes probably from 'entes' (fulfilment, completion) and 'echein,' to have. It is in a way opposite to teleology. In teleologic functions the 'logos' of the 'telos,' the knowledge of the end (the aim) is present. In entelethic processes the character of the result develops through intrinsic forces and the result is only known when reached (unforeseen). An example of the latter is the development of man from ape-like ancestors, who could not have the man-like characteristics as an aim, since these did not yet occur at that time.

This intuitive entelechic junction of engrammata is of a very high order, especially where it concerns our life as individuals and part of the human race.

Consciousness can, on account of the factor of attention (concentration) which plays an important part in it, never see more than one point at a time with sufficient intensity. It cannot survey the reality as an active 'many-in-oneness,' but at the best the natural 'laws' which dominate it, which, however, are also only single threads, and just because they are different and separately illuminated parts of the reality offer a resting-point for the attention.

A law which unites all laws in itself is impossible in our attentive spiritual life. The many-in-oneness of our ego in its environment in completely intuitive or entelechic relation is experienced, therefore, but is not beheld in the attentive consciousness. In the often excellent intuitive judgments much more extensive, especially also more heterogeneous, complexes are sometimes elaborated rather without attention (subconsciously) and mostly these do not become conscious till the final conclusion.

Correlations may also be effected by one single irritation, and manifest themselves in a successive series of imageless instinctive logical actions, just as after the action of fertilization or parthenogenetic irritation of a germ-cell an engrammatic differentiation takes place.

This instinct, not accompanied by conscious images, but manifesting itself in a series of actions, is in some sense an intermediate form between intuition and growth.

The relation of instinct to physical growth may even be very great, as is shown by the fact that in insects special instinctive series of actions coincide according to special seasons and circumstances with phenomena of growth in those seasons.

Indeed, we see logical adaptations in nature, of which it is difficult to say whether they are actions of instinct or growth-phenomena, e.g., in the protrusion of pseudopodia in lower animals.

On the other hand, we see a vicarious action of growth and of instinct. An example of this vicarious action is the way in which statoliths are obtained.

Some animals (the lobster) take them from their environment and lay them in the statocyst, in other animals they grow in it.

Instinct and growth act together and complete each other in such cases, if an animal eats special stuffs instinctively and these change after a series of phenomena of growth into an armor, as in the shell-

formation of bird's eggs. Then instinct and growth, logetical action and logetical formation, become one, both of them based on the logetic entelechy of life.

Since we see this relationship between spiritual life and growth, and we find in both of them associated correlations, memory and entelechy, the question arises, whether in our physical development we can indicate still other factors, which we know play a part in our mental or perceptual life.

I shall discuss still two points here, attention and Weber's law for perceptions.

ATTENTION (CONCENTRATION) IN GROWTH

It appears indeed that in bodily development there occurs something that may be regarded as the spatial transposition of the attention concentrated on one point, viz., the fact that a tissue can only be fully one thing at a time, and the living substance in its specific tissue strives after losing a defective fitness for everything in favor of a concentrated adaptation with regard to one function. This is, however, nothing but the developmental transposition of that which operates as attention in our consciousness. Attention taken in this sense is, just like memory, a general function of organized matter—a functional principle peculiar to the cell in general.

Just as it is I that am attentive and not the attention that is there, so the specificity of a tissue is a specificity which only has significance in the 'many-in-oneness' of the body.

Perhaps we can also explain now by a somatic transposition why the scientific, that is the attentive (discursive, analytic) intellect can have no image of the 'many-in-oneness' of the microcosm or the macrocosm. For conscious conception includes attention, i.e., concentric visioning, and just as a specific tissue which is to-day only a muscle, to-morrow a connective tissue, and the day after tomorrow another thing again, would not form an organ even if it preserves the properties which all the tissues have in common, so the attentive conceptions of our consciousness cannot give us an idea of the world or of life, though we can recognize laws in it as many single threads.

WEBER'S LAW IN PERCEPTIVE AND SOMATIC PROCESSES

It is impossible for me to enter into a discussion at length of the experiments which have been made on Weber's law in connection with phenomena of growth. I will only say that the rather general importance of this law, to which also van Wayenburg has called attention in his thesis, has been confirmed more and more of late.

That the stimulation, which can bring about a modification in situation, forms a constant percentage of the already active influxes, has been shown by Pfeffer for chemotactic, by Massart for heliotropic movements of seed.

Regarding growth it is, of course, very difficult to show that the general growth of a body, either of a plant or animal, experiences arithmetical consequences of geometrically increasing influences, but the chemotropic researches of Miyoshi and the phototropic researches of Miss Wisse, who examined molds, prove that Weber's law holds for specially directed, tropistic phenomena of growth, so that it seems that this law so important in perceptions is to be traced back in growth.

SUMMARY

In summing up the results of my considerations, I come to the following conclusion:

Different factors, which play a part in our conscious experience or in the construction of our mental conceptions, as association, memory, attention, and Weber's law, are conscious realizations of general properties of life, which can be demonstrated equally in the unconscious development of the body.

In our thinking their influence is experienced directly or realized by introspection (in perceptions by the results of certain reflexes); in the growth of the body they appear however 'in concreto.'

Mental life and bodily development are also related in this respect that the unity of our being in both precedes external influences, which influences in both cases activate a primary 'many-in-oneness' (ego).

In mental life the associations in the primary many-in-oneness bring about a secondary many-in-oneness during personal life, which is much less coherent than the primary. Consciousness in consequence of the factor of attention, which always plays a part in it, can never envisage any but units, whether in a spatial sense, such as concrete objects, or abstract units, units in a spiritual sense, such as laws. The many-in-oneness of the ego in its environment, however, is beyond the scope of attentive, scientific consideration, its presence is realized, experienced. Attention operates in it, analyzes it, but does not survey it.

It seems hardly necessary to say that the ideas expressed here are in the main very much in harmony with Aristotle's doctrine concerning the 'psyche' as a general principle of life, which underlies both the rational functions and instinctive actions and phenomena of growth, a conception also defended by Driesch. That also between these three functions there are considerable differences needs no further explanation.

These differences are even so considerable and so evident that they have prevented the greater part of students from seeing the underlying common principles, which, however, by poets are often emphasized (Maeterlinck, for instance, in his '*Intelligence des fleurs*') and also by philosophers like Schelling.

I will not end without a 'plaidoyer' in favor of psychological studies for biological students. It has often appeared to me that this is of great value. Immediate knowledge and the results of introspection must complete our study of the phenomena. Several properties of life—among which the most important—can only be known immediately, not or mainly a posteriori from the study of phenomena.

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Resumen por el autor, Shigeyuki Komine.

Actividad metabólica del sistema nervioso.

IV. La cantidad de nitrógeno no proteínico en el cerebro de las ratas mantenidas en un estado de excitación emocional y física durante varias horas.

Las ratas estimuladas eléctricamente durante un periodo de 10 a 24 horas presentan en su cerebro una cantidad de nitrógeno no proteínico relativamente mayor que el de las ratas normales escogidas como término de comparación. Una estimulación semejante durante 6 horas no aumenta el contenido normal en la rata que "no lucha," pero las que luchan presentan un incremento de productos metabólicos en el cerebro. Las ratas que lucharon violentamente produjeron una cantidad considerable de nitrógeno no proteínico, aun después de una a cuatro horas de estimulación. Las que lucharon durante una hora presentan la cantidad normal de nitrógeno no proteínico en el cerebro después de 42 horas de descanso. El aumento de este nitrógeno en el cerebro como resultado de una lucha violenta, se interpreta como debido en parte a productos metabólicos, que resultan del aumento de la actividad fisiológica general del cuerpo, los cuales llegan al cerebro con la sangre, y, parcialmente también, como el resultado del aumento de la actividad metabólica del mismo cerebro.

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METABOLIC ACTIVITY OF THE NERVOUS SYSTEM

IV. THE CONTENT OF NON-PROTEIN NITROGEN IN THE BRAIN OF THE RATS KEPT IN A STATE OF EMOTIONAL AND PHYSICAL EXCITEMENT FOR SEVERAL HOURS

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Following great emotional disturbances in man, such as fear, horror, or rage, various bodily changes are familiar; for example, cold sweat, the stoppage of saliva, rapid heart beats, trembling, etc. The researches of Pavlov ('10) show beautifully various physiological changes due to the normal activity of the alimentary tract following even insignificant emotional disturbances, and the recent investigation of Cannon and his colleagues ('15) adds to the list of bodily changes, some occurring in connection with the suprarenal glands.

I desired to determine whether there could be revealed any alterations, chemical, physical, or histological, in the brain under such emotional disturbances as are capable of producing the various other bodily changes. So far as I am aware, there are no studies of chemical changes in the brain under a great emotional disturbance. For this reason I undertook to determine as a first step whether or not the content of non-protein nitrogen would change under an altered state of mental activity, or, more precisely, in the state of fear or rage induced when one rat fights with another.

MATERIAL

Albino rats alone were used. The rats were brought into the laboratory from the rat house two or three days before they were tested, and were kept there during this interval in order to accustom them to their new surroundings and with the hope of

eliminating as much as possible the factor of fear from the control rats. The rats were usually fed with a mixture of 'Uneda biscuit' and condensed milk, at about 9 A.M. In making the tests two male rats were put into a box which was constructed in the following manner: A wooden box about 11 inches long, 10 inches wide and 8 inches high was made, in the bottom of which numerous nails were placed with their tips just exposed on the inner surface of the bottom. These nails were connected by means of a copper wire and the ends of these wires were in turn connected with a battery, so that an electric current could pass through them. The rats standing in this box were stimulated for a period of three seconds in every two minutes by the passage of a current. The rats began to fight immediately or shortly after the electrical shock was given, as if one rat held the other responsible for the shock received. Sometimes the rats refuse to fight, and in such cases a light pricking of the tail with a sharp needle always provokes fight almost at once. When once started, the rats continued fighting under the stimulus of the electrical shock alone. Usually the two test rats were taken from different litters, because the rats which belong to the same litter and are accustomed to living in the same cage do not normally fight with each other.

When the rats were brought from the rat house, I put those of the same litter in two separate boxes, one control rat and one test rat in one box, another control and test rat in the other box. Rats of more than 120 days of age were chosen, because Hatai ('17) found that the amount of non-protein nitrogen shows very slight age alteration after the rats pass this age, while on the other hand the rats which are younger do not fight vigorously. Males only were used.

The rats may continue fighting vigorously for several hours. In this operation both rats stand on their hind feet and push each other with their front paws, holding their bodies erect and straight and their mouths almost touching each other. Every time a shock passes both squeak and each pushes the other strongly and they may even bite one another. In some cases the rats continue this performance for more than six hours, while

in other cases they assume a fighting attitude only when a shock passes.

The amount of non-protein nitrogen in the brains of these fighting rats was determined and experiments were also made to determine the amount of non-protein nitrogen in the brains of the rats which had rested for twenty-four hours or more, following a severe fight for a period of one hour. For this latter purpose the stimulated rats were returned to their original cages separately, because such excited rats continue to fight when two of them are placed in the same cage.

TECHNIQUE

The rats were etherized and the blood removed by severing the carotid artery, followed by evisceration. The brain was removed quickly and the left half used for the determination of the non-protein nitrogen, while the right half was taken for a water estimation. From the dried residue the total nitrogen was determined by the usual Kjeldahl method. For the determination of non-protein nitrogen I have employed the method adopted for my former studies on the metabolic activity of the brain ('19); that is, the brain material was finely ground with 2.5 per cent aqueous solution of trichloroacetic acid and then transferred to an Erlenmeyer flask (50 cc.) with a small amount of distilled water. The amount of trichloroacetic solution taken was always twenty times the weight of the sample in grams, while the amount of water used was five times the brain weight similarly expressed, in volume. The mixture of tissue and reagents in the flask is shaken repeatedly during the first hour and then left for twenty-four hours at room temperature. The clear filtrate obtained from this extraction was analyzed by Folin and Farmer's micro-method ('12) as modified by Benedict and Bock ('15). In all cases the nitrogen was estimated by means of the Duboseq colorimeter. The water content of the brain was determined by drying the tissue at 98°C. for one week and the total nitrogen by the usual Kjeldahl method. In this investigation, as in my previous studies, the designation on each flask was replaced by a

conventional mark made by some other member of the laboratory and thus the non-protein nitrogen determinations were conducted in entire ignorance as to which flask belonged to the control or which to the test series, thus avoiding any personal bias in the determinations.

EXPERIMENT SERIES 1

These experiments have been made to see whether or not the amount of non-protein nitrogen in the brain is changed as the result of stimulation (fighting). Altogether six control and six test animals were used. The period of stimulation extended from ten to twenty-four hours. The rats did not fight at all in two cases and only slightly in one. In no instance was the method of pricking with a needle applied to induce fighting. During the experimental period both the control and test animals were not fed except with water. The results are shown in table 1.

As will be seen from table 1, the relative amount of non-protein nitrogen (per 100 grams) in the brain of the test rat is significantly greater than those given by the control rat. The amount of difference is greatest in the rats which had been stimulated for the longest period, but this may be mere coincidence, since the other two cases do not follow in this relation. The present results bring out at least two points. Since these rats were not fed during the period of stimulation, it is conceivable that the electrical shocks, although they did not induce actual fighting, might nevertheless through periodic irritation accelerate metabolic activity as compared with the rats which were not stimulated, and thus produce a form of mild inanition. It has been already found in my previous studies ('19) that during inanition (represented by the later part of the twenty-four-hour period) the non-protein nitrogen content of the brain shows some increase. It will be seen, however, from the later experiments that this increase in the non-protein nitrogen may be mainly due to stimulation, though inanition may also contribute to it.

We might also assume that this increase of non-protein nitrogen in the test brain is due to the increased metabolic activity of

TABLE 1
Showing the amount of non-protein nitrogen, together with other data for the control and test rats

CONTROLS				TESTS			
I	II	III	Average	I	II	III	Average
2	2	2	180	2	2	2	180
324	102	115	1.673	324	102	115	1.713
1.907	1.557	1.554	78.8	1.964	1.509	1.667	78.3
		78.8	2.21			78.3	2.20
		7.96	7.96			8.28	8.20
197	154	176	176	209	170	182	187
				+12	+16	+6	+11
				No fight	No fight	Fought slightly	
				1	2	4	
				10	24	12	15

the nerve cells as the result of electrical stimulation, since we know from the work of previous investigators that the nerve cells show a definite alteration as the result of direct stimulation of peripheral nerves (Hodge, Dolley, and others).

We shall, however, reserve this discussion until further experimental data are presented.

Whatever might be the real cause or causes, we see from this preliminary test that as the result of stimulation the amount of non-protein nitrogen in the brain increases. On account of some defects in our kymograph, it became impossible in the subsequent experiments to run the machine for long periods continuously, and in the later tests we were thus obliged to reduce the maximum stimulation period to six hours.

EXPERIMENT SERIES 2

In the present experiments the test rats were stimulated for six hours with a current from four batteries.¹ Some of these rats did not fight at all, while others made a good fight. When the data are arranged according to the amount of fighting, we obtain interesting results.

As will be seen from table 2, after six hours of stimulation those rats which fought give a significantly greater amount of non-protein nitrogen as compared with the controls, while those rats which did not fight give an amount of non-protein nitrogen almost identical with that for the control brains. It appears from these results that the electrical stimulation alone for a period of six hours is not sufficient to produce a greater accumulation of non-protein nitrogen in the brain, but an emotional disturbance does cause an excessive accumulation of the non-protein nitrogen.

The present experiment seems to indicate that an increased amount of non-protein nitrogen found in the brain of the rats which were stimulated for more than ten hours, and which did not fight at all (experiment 1) might be mainly due to a somewhat increased rate of metabolic activity of the test rats, thus producing a mild inanition. It seems from these data reasonable

¹ Size A, Red Seal Dry Battery, about 30 amperes, when fresh.

to conclude that as a result of great emotional disturbance the circulation of the blood is accelerated, and as a consequence the cell metabolism of the brain is also accelerated, thus producing a greater amount of metabolites in the brain.

EXPERIMENT SERIES 3

Thus far the test rats did not fight vigorously, owing to their lack of response to the electrical stimulus. We found later that when their tails are lightly pricked with a sharp needle they at once begin fighting. By such a simple procedure, accompanied by the electrical stimulus, the rats are made to fight severely, at the same time squealing and biting each other. When once such a violent fight starts the periodic shock is irritating enough to make the fight continue until one rat becomes exhausted and tries to avoid its opponent's attacks. The amount of non-protein nitrogen was determined for those rats which had such a very severe fight for from one to four hours. The results are given in table 3.

The results obtained from the eight independent experiments, using sixteen test rats, show clearly that the amount of non-protein nitrogen in the brain increases as the result of severe fighting when compared with that obtained from the control brain. The amount of non-protein found is, however, irregular and there is no precise indication of a proportional increase with prolongation of the fighting period. In fact, in one instance (the third in table 3) a large amount of decrease is shown as the result of severe fighting for three hours. This decrease in the amount of non-protein nitrogen might have been the result of a complete exhaustion. These irregularities in the amount of non-protein nitrogen found in the brains of test animals may be due to the fact that there are considerable individual differences as to the behavior during experimentation. Some rats are very aggressive and may continue violent fighting without cessation, while there are instances in which the rats fight severely for a few seconds, then stop fighting for some time, only to resume again. Still more important in accounting for the irregularities is the

TABLE 3
Showing the increase of non-protein nitrogen in the brain of the rats which fought vigorously for from one to four hours

CONTROLS									TESTS									
I	II	III	IV	V	VI	VII	VIII	Aver- age.		I	II	III	IV	V	VI	VII	VIII	Aver- age
2	2	2	2	2	2	2	2		Number of rats	2	2	2	2	2	2	2	2	
123	151	122	134	224	239	93	127	152	Age, days	123	151	122	134	224	239	93	127	152
1.746	1.805	1.570	1.548	1.596	1.825	1.505	1.579	1.691	Brain weight, grams	1.775	1.827	1.586	1.698	1.631	1.844	1.618	1.595	1.746
78.3	78.7	78.7	78.4	77.9	78.3	78.6	78.8	78.4	Water, per cent	78.1	78.3	78.5	78.2	78.5	78.5	78.4	78.9	78.4
2.05	2.11	2.00	2.08	2.06	2.10	2.02	2.01	2.05	Total nitrogen, per cent	2.09	2.11	2.09	2.03	2.06	2.07	2.02	2.02	2.06
8.28	7.86	8.27	1.031	8.50	8.45	7.89	9.48	8.57	Non-protein nitro- gen in total ni- trogen, per cent	9.25	8.11	9.10	1.073	9.53	9.15	9.68	8.97	9.18
170	166	216	214	175	184	182	179	184	Milligrams of non- protein nitrogen in 100 grams of brain	198	185	191	217	196	194	196	182	192
									Test differs from control in non- protein nitrogen by	+28	+19	-25	+3	+21	+10	+14	+3	+8
									Response	Fought vio- lently 4	Fought vio- lently 4	Fought vio- lently 4	Fought vio- lently 4	Fought vio- lently 4	Fought vio- lently 4	Fought vio- lently 4	Fought vio- lently 4	
									Stimulus: number of batteries	4	3	3	3	1	1	1	1	
									Hours of stimula- tion	4	3	3	3	1	1	1	1	

fact that some rats show physical exhaustion much quicker than others. It seems to be clear also from the present data that this increase in the amount of non-protein nitrogen in the test brain cannot be the result of inanition, since the period of stimulation is only from one to four hours—mostly one hour—and indeed the increase is often more marked with rats which were stimulated for one hour only.

We may conclude, then, that as the result of violent fighting the amount of non-protein nitrogen accumulates far in excess of that in the control brain, although the exact cause for such an increase is still to be carefully considered.

EXPERIMENT SERIES 4

The experiments so far show clearly that the amount of non-protein nitrogen in the brain increases as the result of stimulation, and it was now thought desirable to determine the effect of rest on the content of the metabolites. For this purpose the rats were induced to fight violently for one hour by methods already described. After the lapse of this period, the test rats were placed separately in the usual laboratory cages and kept there with abundant food and water for from twenty-four to forty-two hours. The results of recuperation for these periods are shown in table 4.

From table 4 it is clear that the amount of non-protein nitrogen in the brain of rats which have rested for twenty-four hours is still significantly higher than those in the control brain. However, in the rats which have rested for forty-two hours the relative amount of non-protein nitrogen is almost the same in both the control and test animals, though the test brains still give a slightly higher value. We might conclude from these data, therefore, that for full recovery to the normal state the rats which have fought violently for one hour require more than forty-two hours' rest. I regret that I cannot extend the observations on resting rats, owing to the limitations of my stay in this country.

TABLE 4

Showing the amount of non-protein nitrogen in the brain of rats which had recuperated for twenty-four to forty-two hours after severe fighting for one hour

CONTROLS							TESTS									
I	II	III	Aver- age	IV	V	VI	VII	Aver- age	II	III	Aver- age	IV	V	VI	VII	Aver- age
2	2	2		2	2	2	2		2	2		2	2	2	2	
125	139	106	123	106	119	113	124	123	138	106	123	106	119	113	124	116
1.612	1.735	1.702	1.683	1.656	1.689	1.665	1.700	1.719	1.815	1.641	1.719	1.629	1.698	1.659	1.665	1.663
78.4	78.0	78.5	78.3	78.6	78.3	78.5	78.4	78.2	77.9	78.3	78.2	78.7	78.8	78.9	78.5	78.7
2.08	2.08	2.02	2.06	2.05	2.06	2.06	2.05	2.08	2.03	2.09	2.08	2.06	2.06	2.04	2.09	2.06
9.80	8.67	9.47	9.31	9.58	9.79	10.06	10.01	9.86	9.76	9.48	9.43	9.24	9.94	10.42	10.05	9.91
203	191	192	192	196	202	208	207	195	198	197	195	190	205	212	209	204
								+3	+17	+5	+3	-6	+3	+4	+2	+1
									Fought vio- lently	Fought vio- lently		Fought vio- lently	Fought vio- lently	Fought vio- lently	Fought vio- lently	
								24	24	24	24	42	42	42	42	42
								4	4	4		4	4	4	4	
								1	1	1	1	1	1	1	1	1

DISCUSSION

From the data presented it seems clear that as the result of severe fighting the amount of non-protein nitrogen increases considerably in the brain. The interpretation of this phenomenon is difficult. In association with violent fighting there is more or less physical exercise, which necessarily accompanies fighting, and we should anticipate an effect of fatigue and whatever changes such fatigue may produce on the brain. Because great emotional disturbance is necessarily associated in this case with marked bodily activity, the greater amount of non-protein nitrogen found in the brain in the present experiment might be considered a result of abnormal physiological activity of various organs and tissues, besides that of the nervous system itself.

The sources of non-protein nitrogen in the central nervous system are two; one is that of the metabolites transported to the brain by means of the blood, and the second is the production of metabolites by the nervous tissue itself. It is, however, impossible to determine from the present experiments alone which of these sources should be held more largely responsible for the greater accumulation of the metabolites in the brain. It is, however, true that the greater activity of the muscles and organs during severe fighting must increase the amount of metabolites in general, and at the same time we are also justified in concluding that the brain tissue itself must increase in its activity. This latter conclusion follows from the investigations of Hodge ('92), which showed that conspicuous structural alterations of the spinal ganglion cells follow the direct electrical stimulation of peripheral nerves. Hodge further demonstrated that the cells of spinal ganglia of English sparrows, of the cerebrum of pigeons, and cerebellum of swallows and antennal lobes of bees obtained at the end of the day, that is, after a period of activity, show structural changes as compared with those obtained at the beginning of the day, or after a night of rest.

Similar observations were made subsequently by several observers, and we may mention here the work of Mann ('95) on the motor cells of the spinal cord and cells of the retina as one illustration.

A series of researches which have been carried on by Dolley ('09, '09 a, '10, '11) show clearly that not only as the result of extreme physical exercises, but even during normal activity, or as the result of surgical shock, the Purkinje cells of the cerebellum show pronounced alterations, not only in structure of the cell body, but in the nucleus-plasma relation. The investigation of Mann ('95) shows clearly the effect of anesthetics on the Nissl granules of nerve cells in producing the so-called chromatolysis, which takes many hours for complete recovery. All these investigations demonstrate that the nerve cells are readily influenced by shock, fatigue, chemical reagents, etc. These cytological alterations of the nerve cells under varied conditions indicate a considerable metabolic activity of the nervous organ, and the increase of non-protein nitrogen in the brain during the great emotional disturbance, which is noted in the present investigation, may thus be regarded as partly the result of activity of the nervous tissue itself. It is the hope of the present writer to further investigate this problem and at least to analyze the non-protein nitrogen bodies here determined into their components (urea, ammonia, amino acids, etc.) in order to throw further light on the source of these metabolites.

CONCLUSIONS

1. The rats which were stimulated electrically for the period of ten to twenty-four hours show a relatively greater amount of non-protein nitrogen in the brain than do the control rats.
2. Similar stimulation for six hours does not increase the normal content in the 'non-fighting' rat, but those rats which do fight show an increase of the metabolites in the brain.
3. The rats which fought violently produced a considerably increased amount of non-protein nitrogen, even after one to four hours of stimulation.
4. The rats which fought severely for one hour show return to the normal content of the non-protein nitrogen in the brain after forty-two hours of rest.
5. The increase of non-protein nitrogen in the brain, as the result of severe fighting, is interpreted as partly due to metab-

olites, resulting from the heightened physiological activity throughout the body in general, and brought into the brain with the blood, and partly as a result of the increased metabolic activity of the brain itself.

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Resumen por los autores, Mathilde L. Koch y Oscar Riddle.

Nuevos estudios sobre la composición química de los cerebros de palomas normales y atáxicas.

Una segunda series de análisis de cerebros de palomas afectadas de una falta hereditaria de regulación en los movimientos voluntarios, demuestra que estos cerebros se distinguen del cerebro normal por el tamaño y composición química. Los cerebros de las palomas atáxicas son mas pequeños. Los autores han hecho ocho análisis de la parte anterior (cerebro) y posterior (cerebro-médula) del encéfalo. Cuatro de estos análisis se llevaron a cabo en palomas atáxicas y los otros cuatro en aves normales de una edad comparable. Las cambios químicos encontrados están más pronunciados en los cerebros de las palomas fuertemente atáxicas que en los de las menos afectadas. También han hecho análisis adicionales de los encéfalos completos de aves muy jóvenes y muy viejas. Los datos sobre los cambios químicos del cerebro que acompañan a la edad han sido obtenidos para una serie de individuos de diversas edades en la paloma. Estos cambios son paralelos a los observados previamente en el hombre y la rata. El exámen de esta "serie de edad" más extensa de cerebros de palomas les ha permitido evaluar mucho mejor que en su trabajo precedente la relación entre las diversas fracciones químicas y la edad. Las diversas fracciones de fósforo y azufre lipoide parecen variar en consistencia con la edad hasta los 600 dias. Una revisión de la significación de los resultados obtenidos en la presente serie de análisis y en la precedente, conduce a la conclusión de que las diferencias observadas indican una escasa diferenciación química o relativa falta de madurez de los cerebros atáxicos. La diferenciación química, que probablemente incluye en parte la mielinización, no procede aparentemente tan deprisa en el encéfalo y, más particularmente, en el cerebelo-médula de los individuos atáxicos como en el encéfalo de los individuos normales.

FURTHER STUDIES ON THE CHEMICAL COMPOSITION OF THE BRAIN OF NORMAL AND ATAXIC PIGEONS

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In an earlier paper ('18) we published the results of five analyses made on the brains of normal and ataxic¹ pigeons. Two of these analyses were of younger and older normal brains; three were of brains affected to three different degrees with an hereditary (Riddle, '18) lack of control of the voluntary movements. Previous observation of the functional derangement led to the conclusion that the seat of the disturbance was probably in the brain.

The five analyses just mentioned supplied some evidence that the functional disorder is associated with deviations from the normal composition of the brain. These deviations or differences we interpreted as indicating a tendency toward infantilism or chemical under-differentiation of the brain of the affected individuals. In other words, the brains of affected individuals of a given age seem chemically more like the brains of normal individuals of a younger age. In that study the number of analyses was not large and the brain (five brains in each analysis) was analyzed entire—without a separation of its parts.

In the present study ten additional analyses were made of brain tissue obtained from birds of still other ages than those previously used. Eight of these analyses were upon samples representing separate portions of the brain—the cerebrum having

¹ In earlier papers the disorder was provisionally called 'ataxia (?)'. In view of the work of Hoshino ('19), mentioned at the conclusion of this paper, and our own present results, it is now perhaps unnecessary to qualify this description of the disease.

been analyzed apart from the rest of the brain (cerebellum and medulla). These ten samples were selected with the purpose of supplementing our previous results in the following respects: *a*) To obtain information concerning the localization (cerebrum or cerebellum-medulla) or non-localization of the previously observed chemical changes in the brain of the affected birds; *b*) a further comparison of the chemical constituents of the ataxic and the normal brain; *c*) the persistence or non-persistence of such differences in older birds; *d*) the extension of our knowledge of the relation of age² to the chemical composition of the brain.

MATERIALS AND METHODS

The brains used in the preparation of samples I, II, III, IV, and VI are from birds similar to those used in our previous study except for age differences. The two groups of ataxic birds showed the disorder to different degrees. The older group (sample III) being clearly the more affected.³ The birds which supplied the material for sample VI were considerably younger than the birds used in the earlier study, while the other four samples were obtained from somewhat older birds (II and IV), and from much older (I and III) birds. The birds used in the preparation of sample VI were mostly too young to classify as normal or ataxic.

All of the above-mentioned birds, like those used for the previous study, were birds descended from the first obtained ataxic or affected individual. These birds, ataxics and normals, were therefore considerably inbred. The normals or 'controls' of these groups were of the same strain and parentage as the ataxics; they were, in the main, brothers and sisters of the ataxics. Sample V contained the brains of the oldest common pigeons (mostly homers) of the same general kind, but without ataxic blood, which we could obtain from our collection.

In the present study the cerebrum was analyzed separately from the cerebellum-medulla in four cases; i.e., four groups of

² Precise information of this sort has been obtained hitherto, so far as we are aware, only in man and in the rat. The data for man are very incomplete.

³ The reader should consult our earlier papers for adequate descriptions of the various manifestations or degrees of manifestation of the ataxia.

brains yielded materials for eight analyses. The two additional analyses, samples V and VI, were of entire brains, although here also the cerebrum was weighed separately from the rest of the brain.

The birds were all killed by decapitation and the brain removed immediately, using the following technique: After removing the feathers and skin, the skull was opened at the posterior end. The dorsal surface of the medulla and cerebellum was exposed up to the point of the anterior border of the cerebellum by removal of the skull and meninges. The cerebellum was then turned back until the posterior border of the optic lobes was exposed. The separation of cerebellum and anterior region of the brain was affected by cutting just posterior to the cerebral peduncles and the posterior border of the optic lobes. The medulla and cord were severed at the foramen magnum and the posterior brain weighed (between watch-glasses) immediately.

The remainder of the dorsal and anterior skull was next removed. The olfactory nerves were cut and the cerebrum turned back so as to expose the optic chiasma. The optic nerves were severed about 1 mm. anterior to the chiasma. The cerebrum was removed by tilting it backward and cutting the cranial nerves close to the brain. It was then immediately weighed (between watch-glasses) and placed in a sufficient quantity of redistilled alcohol to make the final concentration of alcohol about 85 per cent. Analysis was begun two months after the collection of the material.

The method used in the analysis of this material is that of Waldemar Koch ('09) and the same⁴ as was used in the previous study.

PRESENTATION OF DATA

Our earlier work with the brain of the pigeon made it evident that it is necessary to obtain data on the age, sex, body weight, and normality or abnormality of each bird whose brain was collected for chemical analysis. These data for the birds used in

⁴ The method has been recently republished with slight modification by M. L. Koch and C. Voegtlin ('16).

the present study are given in tables 1 to 3. The weights of the two parts of the brain, the weight of the entire brain, the weight of body, the sex, and the age of the birds of the group are included in the same tables. In these tables the birds are

TABLE 1

Details on the materials used in the preparation of the normal (control) pigeons' brains

	NUMBER OF BIRD	SEX	BODY WEIGHT	BRAIN WEIGHT			AGE
				Cere- bellum and medulla	Cerebrum	Whole brain	
Older normals (sam- ples I and Ia)	B530	♂	307	0.445	1.435	1.880	<i>days</i> 887
	B523	♂	352	0.457	1.563	2.020	820
	B548	♂	344	0.465	1.500	1.965	783
	B665	♂	376	0.466	1.413	1.879	722
	B489	♂	372	0.460	1.423	1.883	674
	K288	♂	315	0.450	1.518	1.968	564
	K112	♀	315	0.395	1.385	1.780	432
	K178	♂	340	0.505	1.471	1.976	414
	K217	♀	305	0.462	1.364	1.826	392
	E232	♂	304	0.447	1.406	1.853	298
Average.....			334.0	0.4552	1.4478	1.9030	598.6
Younger normals (samples II and IIa)	K251	♀	225	0.418	1.360	1.778	294
	K239	♀	316	0.403	1.418	1.821	290
	K284	♂	291	0.482	1.573	2.055	281
	K235	♀	288	0.465	1.374	1.839	262
	K265	♀	293	0.435	1.333	1.768	255
	K250	♂	313	0.492	1.486	1.978	219
	M364	♂	291	0.503	1.400	1.903	169
	M366	♂	352	0.520	1.507	2.027	129
	M471	♂	322	0.487	1.443	1.930	80
	M430	♀	274	0.420	1.358	1.778	76
Average.....			296.5	0.4625	1.4252	1.8877	205.5

all arranged according to decreasing age. These tables are given in order that all of the necessary data may be presented, including the size and composition of each sample as prepared for analysis. These tables are not used directly in the comparisons made below.

Relations of sex, body weight, and age to brain weight (table 4)

When the data concerning the six bird and brain groups of the above tables are subdivided on the basis of sex and arranged in

TABLE 2

Details on the materials used in the preparation of the ataxic (affected) pigeons' brains

	NUMBER OF BIRD	SEX	BODY WEIGHT	BRAIN WEIGHT			AGE <i>days</i>
				Cere- bellum and medulla	Cerebrum	Whole brain	
Older ataxics (sam- ples III and IIIa) (More ataxic)	A436	♀	249	0.395	1.541	1.936	943
	B565	♀	284	0.404	1.431	1.835	820
	B661	♀	318	0.391	1.328	1.719	731
	B615 ¹	♀	335	0.386	1.224	1.610	701
	B492	♀	366	0.393	1.280	1.673	673
	K105	♂	330	0.424	1.520	1.944	537
	K139	♀	332	0.414	1.393	1.807	511
	K198	♂	263	0.430	1.315	1.745	398
	K169 ¹	♀	342	0.453	1.303	1.758	392
	K292 ²	♂	268	0.462	1.503	1.965	298
Average.....			308.7	0.4152	1.3838	1.7992	600.4
Younger ataxics (samples IV and IVa) (Less ataxic)	K274	♀	282	0.410	1.470	1.880	285
	K269	♀	254	0.373	1.390	1.770	283
	K255	♀	267	0.417	1.382	1.799	275
	K241	♀	261	0.403	1.325	1.728	258
	K248	♀	252	0.426	1.365	1.791	258
	K293	♂	293	0.444	1.585	2.029	220
	M369	♂	295	0.495	1.478	1.973	187
	M398	♂	258	0.473	1.532	2.005	136
	M459 ³	♂	202	0.400	1.430	1.830	79
	M429 ³	♂	173	0.442	1.269	1.711	79
Average.....			253.7	0.4283	1.4233	1.8516	206.0

¹ Very scraggly feathers.

² This bird ataxic when younger; practically normal when killed.

³ Somewhat emaciated.

order of age, a number of interesting facts are made clear. It may first be noted that in every case the males of a group show a larger average brain weight than that of the females of the group.

Indeed, in the four really comparable⁵ groups the lowest average brain weight for males is higher than the highest average for females. The same is true for the two separate parts of the brain—the male cerebellum-medulla is larger and the male cerebrum is larger.

That this larger size of the brain, and of both parts of the brain, of the male is not wholly dependent upon the larger body size of the male is indicated by the fact that two only of the male

TABLE 3

Details on the materials used in the preparation of groups V and VI

	NUMBER OF BIRD	SEX	BODY WEIGHT	BRAIN WEIGHT			AGE
				Cere- bellum and medulla	Cerebrum	Whole brain	
Older pigeons of other strains (sam- ple V)	H-A	♂	435	0.438	1.527	1.965	<i>days</i> 3266
	H-B	♂	394	0.511	1.535	2.046	3264
	169	♀	334	0.510	1.528	2.038	1287
	A21	♀	335	0.472	1.523	1.995	1238
	A313	♀	258	0.457	1.505	1.962	1048
Average.....			351	0.4776	1.5236	2.0012	2021
Younger pigeons of the ataxic strain (sample VI)	M475	♀	316	0.442	1.352	1.794	59
	M452	♀	163	0.393	1.223	1.616	59
	M478	♂	255	0.454	1.384	1.838	52
	M479 ¹	♂	319	0.427	1.323	1.750	50
	M441	♀	264	0.367	0.941	1.308	37
	M408	♀	129	0.335	0.940	1.275	35
	M415	♀	75	0.198	0.542	0.740	22
Average.....			217.3	0.3737	1.1007	1.4744	45

¹ Known to be ataxic.

groups are larger and two are smaller than the associated females. For the mature birds the brain weight shows considerable independence of body weight.

The relation of age to the size of the brain and to each of the two parts into which we have divided it can be partly under-

⁵ Group VI is clearly immature; group V is not of the same strain. In the latter group, moreover, the disparity of age is extreme; the males being very old (nine years) and the females in their prime (three years).

TABLE 4
The relation of sex to size of pigeons' brain, body weight, and age. Listed separately for each sample as prepared for analyses (from tables 1 to 8)

NUMBER OF SERIES	DESCRIPTION	SEX	BODY WEIGHT		BRAIN WEIGHT				RATIO OF PARTS ¹	AGE	
			Mean of $\sigma - \phi$		Cerebellum and medulla		Cerebrum			Whole brain	
			Average	Mean of $\sigma - \phi$	Average	Mean of $\sigma - \phi$	Average	Mean of $\sigma - \phi$		Average	Mean of $\sigma - \phi$
V	Oldest birds ²	$\left\{ \begin{array}{l} 2\sigma \\ 3\phi \end{array} \right.$	415 309	0.475 0.480	0.475 0.4775	1.531 1.519	1.525 1.998	2.006 2.0035	1:3.22 1:3.17	3275 1191	days 2233
I-Ia	Older	$\left\{ \begin{array}{l} \text{Normals} \\ \text{Ataxics}^3 \end{array} \right.$	340 310	0.462 0.429	0.4455	1.466 1.375	1.4205	1.928 1.803	1:3.17 1:3.20	645 421	533
III-IIIa		$\left\{ \begin{array}{l} 3\sigma \\ 7\phi \end{array} \right.$	287 318	0.439 0.405	0.4220	1.446 1.357	1.4015	1.885 1.762	1:3.29 1:3.35	411 632	546.5
II-IIa	Younger	$\left\{ \begin{array}{l} \text{Normals} \\ \text{Ataxics}^3 \end{array} \right.$	314 279	0.497 0.428	0.4525	1.482 1.369	1.4255	1.979 1.797	1:2.98 1:3.19	176 235	204.5
IV-IVa		$\left\{ \begin{array}{l} 5\sigma \\ 5\phi \end{array} \right.$	244 263	0.451 0.406	0.4285	1.459 1.388	1.4235	1.909 1.794	1:3.23 1:3.42	140 272	206
VI	Youngest birds ⁴	$\left\{ \begin{array}{l} 2\sigma \\ 5\phi \end{array} \right.$	287 189	0.441 0.347	0.3970	1.354 1.000	1.177	1.794 1.347	1:3.07 1:2.88	51 42	46.5

¹ Ratio of weight of cerebellum-medulla to weight of cerebrum.

² No ataxic 'blood' in these birds.

³ Figures for this extremely ataxic group are written in italics.

⁴ Probably some ataxics and some normals in this group (too young to classify); all birds of ataxic 'blood' or strain.

stood from a study of the data of table 4 and partly from the data previously obtained by us ('18, table 3; in part reproduced here, table 8). It is clear that neither females of 42 days nor males of 51 days (averages on table 4) have fully developed brains. Two females of 69 and 127 days ('18, table 3), however, each had a brain nearly as large (1.811 grams and 1.813 grams) as that of the largest female brain of groups I and II (K235 = 1.839 grams, age 262 days, table 1) and larger than the average brain (1.803 grams and 1.797 grams, table 4) of the females of these much older normal groups. Similarly, a male of 124 days ('18, table 3) had a brain larger (1.943 grams) than the average (1.928 grams) of eight normal males of 645 days (average, table 4).

It is reasonably clear that in this particular strain of birds the maximum brain weight is usually attained not much later than 100 days after the beginning of development (eighteen days for incubation). In all of our present and previous analyses of pigeon brains (table 8), therefore, only the brains of group VI of the present series were undersized because of age. Groups II and IV, which are compared with each other, have each two birds aged less than 100 days.

Relation of ataxia to brain size

The relation of brain size to normality and ataxia may now be confidently studied, since the influence of sex, body weight, and age have already been considered. Four quite comparable groups (I to IV, table 4) are available; two of these are brains from normal birds and two from ataxics, and there are both males and females in each of the four groups for comparison. The following is found:

The *whole brain* of each of the ataxic groups is smaller⁶ than that of either of the two normal groups (tables 1 and 2). The

⁶ There is a high percentage of males in one normal group and a high percentage of females in one ataxic group which considerably affects the brain size of these two. But the comparison between the normal and ataxic males of these two groups, and between the normal and ataxic females of these two groups, is just as valid as are the similar comparisons between the other two groups in which no disparity of sex exists. The mean weights of the various groups permit a quite fair comparison from one group to another.

males of both ataxic groups have smaller brains (table 4) than have the males of either normal group. The females of both ataxic groups have smaller brains than the females of either normal group.

The *cerebrum* of all of the above-mentioned groups and subdivisions of groups of ataxics is smaller than the corresponding groups of normals in a precisely similar way except that the females of the less ataxic group have a larger cerebrum than do the females of the other three groups. The females of the strongly ataxic group have the smallest cerebrum found for the four groups.

The *cerebellum-medulla* of all the ataxic groups and subdivisions is smaller in every case.

Further study of these data (four comparable age and strain groups of table 4) shows, moreover, that the posterior portion of the brain (*cerebellum-medulla*) of the ataxic groups is disproportionately small in comparison with the cerebrum. That is to say, the cerebrum of ataxics is somewhat reduced (1.5 per cent in males and 0.0 per cent in females) below that of the normals, while the *cerebellum-medulla* is much below (7.2 per cent in males and 5.4 per cent in females) the normal size. The mean weight of the cerebrum of the older ataxics is 1.3 per cent below that of the older normals. That of the younger ataxics 1.7 per cent below that of the younger normals. For the *cerebellum-medulla* these figures are 5.3 and 5.5 per cent, respectively. The disproportionate decrease of the ataxic *cerebellum-medulla* is also shown by the figures for the 'ratio of parts' of the brain. These figures are given in the next to the last column of table 4. All of the (four) subdivisions of ataxics are there shown to have abnormally small posterior brains. These same ratios also demonstrate that in all of the four comparable groups—normals and ataxics—the *cerebellum-medulla* is a smaller fraction of the total brain in females than in males.

It follows, therefore, that ataxia carries the male brain in the direction of the normal female brain, both in regard to size and relative proportion of its parts. We have hitherto noted that ataxia also reduces the body size of the male; this is again in the

direction of the normal female. It will be pointed out later that ataxia is found more often in females than in males. Since the observed effects of ataxia on the male all take the direction of the female, it may be asked, does this fact have any bearing upon the predominant appearance of the derangement in female offspring?

Before concluding the above considerations (in which the materials entering into the composition of the samples are being considered as fully as a paper presenting chemical data permits), emphasis may be placed upon the fact that samples I-Ia and III-IIIa (older normals and older ataxics), though quite comparable as to age, are not so in regard to sex. Also, that this sex difference at least partially accounts for the size differences of the brains of these two groups. And, further, that differences of brain size may be of significance in the results of the chemical analysis. Donaldson ('16) obtained from the rat evidence "that both the relative and absolute weight of the brain * * *, at a given age, are factors tending to modify the percentage of water present, in the sense that the heavier brain or cord shows the smaller percentage of water." Donaldson⁷ also indicates that in a given species the larger (heavier) brains at a given age tend to have a higher percentage of white substance. On this basis the larger brains of both the older and younger normals (samples I-Ia, II-IIa) might be expected to show lower water values (and other chemical evidences of greater age) than the ataxic groups of somewhat smaller brain size with which they are compared. Possibly such size relations do slightly influence the amount of the various chemical fractions obtained by us. We would note, however, that the cerebrum of the younger normals (IIa) and the younger ataxics (IVa) were of equivalent (total) size (14.252 grams and 14.233 grams, tables 1 and 2), and in each sample the sexes were equally represented; nevertheless, when the figures obtained for these two groups are compared, on the basis of the nine constituents found to be characteristic of age in this series (p. 98), it is found that six of these nine constituents here indicate the relative immaturity of the ataxic

⁷ Personal communication.

group. Further, until exact information as to the nature of the changes in the (smaller) ataxic brains are made known by neurological study (Hoshino), it does not seem practicable or profitable for us to attempt to evaluate the influence of the brain-size differences, which are present in some of the samples, upon the chemical data obtained by us.

Chemical criteria of under-differentiation or immaturity in pigeon brain

In the presentation of our earlier results ('18) we endeavored to make a comparison of the observed chemical differences of ataxics and normals in terms of known or expected changes due to age. It seems advisable to follow the same plan in the present paper. At the time of our earlier publication we had only the five different ages (only two of which were brains of normal birds) represented in our own analyses to guide us as to the actual nature and direction of chemical changes due to age in the pigeon brain. The brains utilized by us ranged between the relatively narrow limits of 106 days and 183 days. As a check and as a more complete guide to the direction followed by chemical change in brain tissue with increasing age, we utilized (and freely quoted) two available series of results on other animals. Human brains (Koch and Mann, '07) aged six weeks, two years, and nineteen years, and rat brains (W. Koch and M. L. Koch, '13) aged one to 120 days—all of which were analyzed by methods essentially the same as those used by us—were our only additional guides.

Realizing the need for specific and positive knowledge of the course of chemical differentiation in the pigeon brain in still younger and in much older ages in our present study, we have examined the brains of normal birds aged (averages) 45, 205, 598, and 2,021 days. As a result of these additional analyses, we can now see that several of the most pronounced chemical changes, which elsewhere are known to accompany increased age in brain tissues, were largely completed in the youngest of the brains utilized in our former study. And further, it has now become plain that some chemical fractions (extractives, sulphatids, and

phosphatids) which are really indicative of age in very young brains have very limited or quite uncertain values when applied to brains of some of the older ages. This particularly applies to several ages actually studied by us. It is, therefore, necessary to restate here the chemical criteria for differentiating younger and older stages, as this applies to the pigeon brain for those particular ages which we are now to compare.

The data given in table 8 require the following conclusions:

Water is decreased relative to solids throughout the entire age series. It is true that the moisture figures obtained for the several groups of normals do not correctly indicate the age of the group in all cases. For example, the normal brain of 106 days is shown to have a slightly lower percentage of water than the normal brains of 205 days, and an ataxic of 133 days slightly less water than an ataxic of 206 days. Ataxia itself probably further complicates the smoothness of the figures for the series as a whole. Nevertheless, a general tendency to a decrease of water with increasing age is unquestionable.⁸

Protein plainly decreases with increased age. Only the figure for the normals of 106 days breaks the complete smoothness of the curve for the entire series of normals.

Lipoids increase with increased age, although the figures actually obtained are not wholly consistent, neither for the normals considered alone nor for the ataxic series. In fact, between 106 days and 598 days very little change is indicated in the amount of lipoids. This doubtless indicates that myelination is practically completed in these pigeons at 106 days.

Extractives are present in the solids in greater amount in the forty-five day brain than at any other time. In normals of 106 days, however, no more extractives are present than in normals of 2,021 days, and less is found than in normals of 205 and 598

⁸ It should be borne in mind that there is opportunity for error in the moisture estimation of any organ such as the brain. First, through unequal evaporation from the brain surface during the preparation of the sample, and, second, through the presence of unequal quantities of blood within the organ when weighed. Whether ataxia itself offers any special complication is at present unknown to us. Again, any loss or gain to the solids of either the alcohol-ether soluble or alcohol-ether insoluble fraction would serve to modify the recorded amount of moisture.

days. The smallest percentage of extractives was found in normals of 183 days. It is therefore clear that in brains older than 106 days the variations in amount of extractives noted by us have a wholly doubtful significance with respect to age.

Cholesterol steadily increases with age. The relative age of all except one of the normal groups is correctly expressed by the amount of cholesterol found.

Phosphatids increase with age (total phosphorus, in per cent of solids, steadily decreases with age) until about 205 days. The amount then decreases slightly. It is probable that low phosphatids, as in birds of 600 days,⁹ is indicative of relative immaturity, since it is only in very immature brains that low phosphatids are normally found. The highest figure obtained was for a group of 183 days.¹⁰ In general, therefore, phosphatids cannot be considered distinctive of age in brains older than 183 days.

Sulphatids certainly increase with increased age (total sulphur, in per cent of solids, fluctuates with age) to 205 days or more; but the figures obtained, like those for phosphatids, are not entirely consistent. The highest figure for sulphatids was obtained in brains of 205 days. It seems probable that the percentage of sulphatids is actually higher in the cerebrum of birds of about 205 days than in those of about 600 days (table 5). Although the series as a whole indicates that lower sulphatids signifies younger age, we do not seem warranted in applying this rule to brains of 200 to 600 days old. (This is in no way contradictory to W. Koch's conclusion that phosphatids and sulphatids increase in the brain of the growing animal.)

Phosphatids and sulphatids, however, require a further remark. When the amounts of phosphatids (lipoid-phosphorus) and sulphatids (lipoid-sulphur) are calculated respectively in terms of percentage of total phosphorus and total sulphur (table 7), the

⁹ The cerebellum-medulla of 598 and of 600 days constitute a further exception. These have less lipid-phosphorus than their corresponding (normal and ataxic) groups of 205 and 206 days (table 5). Possibly in the pigeon the cerebellum-medulla is chemically a more fully differentiated 'brain tissue,' and attains its chemical differentiation earlier than the cerebrum.

¹⁰ A similar situation has been found for the brain phosphatids of the rat (Koch and Koch, '13).

above-stated abnormal relation of phosphatids and sulphatids to age (at 200 to 600 days) disappears. The difference of result on this basis of calculation is directly due to the fact that total

TABLE 5

Chemical composition of cerebrum and cerebellum-medulla of normal and ataxic pigeons (in per cent of solids)

GROUP	CEREBRUM				CEREBELLUM AND MEDULLA			
	IIa. Normals, 205 days	IVa. Ataxics, 206 days	Ia. Normals, 598 days	IIIa. Ataxics, 600 days	II. Normals, 205 days	IV. Ataxics, 206 days	I. Normals, 598 days	III. Ataxics, 600 days
	Younger		Older		Younger		Older	
Water in per cent.....	81.0	80.3	79.7	79.6	77.9	78.0	77.9	77.8
Proteins.....	52.1	51.3	49.9	50.3	46.3	46.8	45.8	46.2
Lipoids.....	34.4	35.3	35.8	35.0	40.7	41.4	41.9	41.8
Extractives.....	13.5	13.4	14.3	14.7	13.0	11.8	12.3	12.0
Cholesterol.....	7.5	7.4	7.4	7.3	8.8	8.8	9.0	8.9
Phosphatids.....	22.5	22.5	22.3	20.2	24.6	23.4	22.5	23.0
Sulphatids.....	8.4	8.4	6.8	5.6	13.3	11.3	11.8	15.9

Distribution of sulphur in per cent of total sulphur

Protein-sulphur.....	65.3	69.7	66.6	69.9	55.8	54.5	56.8	49.6
Lipoid-sulphur.....	18.1	16.5	18.9	15.3	26.6	23.5	27.2	32.1
Extractive-sulphur.....	16.6	13.8	14.5	14.8	17.6	22.0	16.0	18.3
Total sulphur (in per cent of solids)	0.93	1.02	0.72	0.76	1.00	0.96	0.87	0.97

Distribution of phosphorus in per cent of total phosphorus

Protein-phosphorus.....	17.7	20.1	13.9	16.0	18.7	18.0	17.9	20.4
Lipoid-phosphorus.....	63.0	59.8	67.5	62.1	61.7	60.2	61.2	59.3
Extractive-phosphorus.....	19.3	20.1	18.6	21.9	19.6	21.8	20.9	20.3
Total phosphorus (in per cent of solids)	1.39	1.46	1.28	1.26	1.55	1.50	1.43	1.51

phosphorus and total sulphur are reduced in the brains of about 600 days. Calculated thus, lipoid-phosphorus is in greater amount in the 598-day normal than in the 205-day normal.

Also, lipid-sulphur is in greater amount in the 598-day than in the 205-day brain. In the comparison of the brains of these ages soon to follow (table 5), we shall therefore use the figures obtained for lipid-phosphorus and lipid-sulphur (table 6) and ignore the figures for phosphatids and sulphatids which are calculated by factor and in terms of per cent of solids.

The other fractions (protein and extractive) of sulphur and phosphorus may next be considered. They, too, are calculated (table 7) in per cent of total sulphur and of total phosphorus.

TABLE 6

*Distribution of sulphur and phosphorus in cerebrum and cerebellum-medulla
(calculated in per cent of solids)*

GROUP	CEREBRUM				CEREBELLUM AND MEDULLA				
	Sulphur								
	Protein	Lipoid	Extrac- tive	Total	Protein	Lipoid	Extrac- tive	Total	
Older.....	{ Ataxics Normals	0.528	0.111	0.116	0.755	0.481	0.310	0.177	0.968
		0.479	0.136	0.104	0.718	0.494	0.235	0.139	0.868
Younger....	{ Ataxics Normals	0.708	0.167	0.140	1.015	0.526	0.226	0.213	0.964
		0.609	0.169	0.155	0.933	0.559	0.267	0.177	1.003
		Phosphorus							
Older.....	{ Ataxics Normals	0.202	0.783	0.276	1.262	0.308	0.894	0.307	1.509
		0.178	0.867	0.239	1.284	0.256	0.874	0.298	1.428
Younger....	{ Ataxics Normals	0.293	0.872	0.293	1.458	0.272	0.910	0.330	1.512
		0.245	0.873	0.268	1.387	0.288	0.956	0.304	1.548

Protein-sulphur is present in largest amount in brains 166 and 183 days old, and in lowest amount at 205 days. It is lower in the three oldest groups than in the three youngest groups;¹¹ but the irregularity just noted makes it impossible to use this fraction as an index of age. *Extractive-sulphur* and *total sulphur* plainly do not vary consistently with age.

¹¹ Protein sulphur is lower in both cerebrum and cerebellum-medulla of normals of 598 days than in normals of 205 days.

Protein-phosphorus decreases wholly consistently with age in all of the normals. *Extractive-phosphorus* decreases progressively with age. This rule fails, however, in the very old (2,021-day) brain. *Total phosphorus* also progressively decreases with age.

In the comparison of the normal and ataxic brains, the younger age is characterized, therefore, by higher values for water, protein, protein-phosphorus, extractive-phosphorus, and total phosphorus; and by lower values for lipoids, cholesterol, lipid-phosphorus, and lipid-sulphur. A comparison, on the basis of these nine constituents, of corresponding parts of the brain of normals and ataxics will be made first. That of the whole brain of all of the normals and ataxics can be better done later.

Results of analysis of cerebrum and cerebellum-medulla of normals and ataxics (table 5)

The cerebrum of the younger (less) ataxic group gave lower figures for moisture, protein (extractives),¹² cholesterol, lipid-sulphur, and lipid-phosphorus than the younger normals with which they should be compared. Higher figures were obtained for lipoids, protein-phosphorus, extractive-phosphorus, and total phosphorus. Six of these figures indicate that the cerebrum of the younger ataxics (206 days) were less differentiated than those of the younger normals (205 days); three figures point to the opposite conclusion.

The cerebellum-medulla of the younger ataxics show smaller values for lipid-sulphur, protein-phosphorus, lipid-phosphorus (phosphatids, sulphatids, extractives), and total phosphorus; greater values for moisture, protein, lipoids, and extractive-phosphorus. Five of these figures point to the (less) ataxic cerebellum-medulla as the younger stage, while three are opposed. Cholesterol shows no difference. Summarizing this comparison of parts of the brain of younger normals and younger (less) ataxics, it may be said that the results show but little of chemical

¹² Substances which are not really distinctive of age will sometimes be included in the summaries or comparisons which follow, but to distinguish them they will be included in parentheses.

difference which is consistently interpreted on the basis of age. The differences found, however, favor the view that both the cerebrum and cerebellum-medulla of the ataxics were somewhat younger than the normals with which they are compared. In reality, our observed moisture differences of 0.1 per cent are insignificant.

The cerebrum of the older (strongly) ataxic group show decreased water, lipoids, cholesterol (phosphatids, sulphatids), lipoid-sulphur, lipoid-phosphorus, and total phosphorus, when compared with the amounts found in the older normal group. Increased values are shown for protein (extractives), protein phosphorus and extractive-phosphorus. Seven of these figures indicate juvenility or chemical under-differentiation of the ataxics as compared with the normals of equivalent age. Two figures, those for the very nearly equivalent water and total phosphorus, oppose this interpretation. Reference to table 4 will show that the cerebrum in this group of ataxics was below normal size.

The cerebellum-medulla of the older ataxics show decreased amounts of water, lipoids (extractives), cholesterol, lipoid-phosphorus, and extractive-phosphorus, and increased amounts of protein (phosphatids, sulphatids), lipoid-sulphur, protein-phosphorus, and total phosphorus. Of these figures, six are in favor of, and three are opposed to, the view that the cerebellum-medulla of the ataxic group is more juvenile than that of the normal group.

Most of the chemical evidence which is distinctive of age indicates, therefore, that both parts of the brain of the older group of strongly ataxic birds (600 days) were somewhat less old than the older normal brains (598 days) with which they must be compared. Similar evidence was found for both cerebrum and cerebellum-medulla of the younger (less) ataxic group.

Concerning the whole of the new evidence obtained by a comparison of the chemical composition of the parts of the brain of normals and ataxics, it can be said that all of the four tests made, support the interpretation which was given to our previous results. Most of the evidence indicates that the cerebrum and cerebellum-medulla of both ataxic groups are chemically less dif-

ferentiated, or less old, than are these parts of the brain in normals of equivalent age. Further, the evidence obtained from the older strongly ataxic brains is more decisive than that obtained from the younger less ataxic brains.

Distribution of sulphur and phosphorus in cerebrum and cerebellum-medulla

In table 6 are given the data on the distribution in cerebrum and cerebellum-medulla of sulphur and phosphorus calculated in per cent of solids. That method of calculation scarcely changes¹³ the description already given above in terms of total sulphur and total phosphorus. Particular attention may be directed only to differences in distribution of these elements in the cerebrum and cerebellum-medulla. These data are the first thus far obtained for any bird.

Protein-sulphur is more abundant in the cerebrum than in the cerebellum-medulla. Lipoid-sulphur and extractive-sulphur is distinctly less in the cerebrum. The older birds (598 and 600 days) have markedly less sulphur in all fractions of the cerebrum than have the younger birds (205 and 206 days). In the cerebellum-medulla there is less of difference due to age. This probably indicates that the maximum sulphur content of the pigeon cerebrum is reached at nearly 206 days and thereafter decreases in relative amount (table 7). The sulphur of the cerebellum-medulla suffers no marked decrease during this period (206 to 600 days). Most of the sulphur of cerebrum and cerebellum-medulla is protein-sulphur.

The phosphorus of both the cerebrum and the cerebellum-medulla is chiefly lipoid-phosphorus. Protein-phosphorus and extractive-phosphorus are present in almost equal quantity in both parts of the brain. All three fractions of phosphorus are

¹³ Only two of the figures compared above show a different relation to each other under the two methods of calculation. These occur in the lipoid-phosphorus and extractive-phosphorus of the cerebellum-medulla of the older ataxic group. Both became higher in the ataxic than in the normal. The numerical result is the same as before: six figures still indicate the relative immaturity of the organ and three figures are opposed.

present in slightly greater amounts in the cerebellum-medulla than in the cerebrum.

The distribution of sulphur and phosphorus, calculated for the entire brain of the four bird groups considered above, is shown in table 7. These figures are of course based upon the original actual weights. Similar figures for brains of 45 days and 2,021 days of the present series of analyses, besides corresponding figures from our five previous analyses, are included for comparison. Reference to the data of this table has already been made.

TABLE 7

Distribution of sulphur and phosphorus in per cent of total sulphur and total phosphorus for whole brain of all analyses

NUMBER	GROUP	AGE	SULPHUR				PHOSPHORUS			
			Protein	Lipoid	Extrac- tive	Total ¹	Protein	Lipoid	Extrac- tive	Total ¹
		<i>days</i>								
1	Normal	2021	60.2	23.5	16.3	0.84	14.1	61.7	24.2	1.40
2	Ataxic	600	63.9	19.9	16.2	0.81	17.3	61.3	21.4	1.32
3	Normal	598	63.8	21.3	14.9	0.76	15.0	65.9	19.1	1.32
4	Ataxic	206	66.0	18.2	15.8	1.00	19.5	59.9	20.5	1.47
5	Normal	205	62.6	20.5	16.9	0.95	18.0	62.6	19.4	1.43
6	Normal	183	69.6	18.2	12.1	0.69	19.0	60.8	20.3	1.50
7	Ataxic	166	69.9	18.2	13.9	0.75	18.1	59.2	22.7	1.51
8	Ataxic	158	67.9	16.7	15.4	0.77	18.5	58.9	22.6	1.44
9	Ataxic	133	65.1	(21.2)	13.7	(0.76)	19.5	58.4	22.1	1.49
10	Normal	106	65.8	19.4	14.8	0.67	19.3	58.2	22.5	1.53
11	Mixed	45	65.2	11.4	23.4	0.73	20.2	55.1	24.8	1.61

¹ In per cent of total solids.

NOTE.—Nos. 1 to 5 and 11 are new data; nos. 6 to 10 are our earlier data ('18).

Summary of present and earlier data on chemical differences in ataxic brains

In tables 7 and 8 are given for the whole brain the principal analytical figures obtained by us in the present and former series of analyses. Samples V and VI (nos. 1 and 11 in these tables) and samples I–IV and Ia–IVa (nos. 2 to 5) of the present series are there calculated for the whole brain.

In these tables the composition of the most ataxic brains of the present and former series, nos. 2 and 8, respectively, may be

readily compared (figures for both placed in *italics*) with the brains of similar ages. It is in these two series in which the abnormality was most marked that the clearest evidence for a chemical under-differentiation or relative immaturity of the ataxic brains is found. It is notable that in both of these groups the amount of water is either equivalent to or more than is indicated for their actual age; protein is present in excess in both; lipoids are deficient in both; cholesterol is lowest in both; phosphatids and sulphatids¹⁴ are also low in both; total phosphorus

TABLE 8

Chemical composition of the whole brain of normal and ataxic pigeons (in per cent of solids). Arranged according to age

NUMBER	DESCRIP- TION OF GROUPS	AVERAGE			WATER	SOLIDS			CHOLE- STEROL	PHOS- PHA- TIDS	SULPHA- TIDS
		Age	Body weight	Brain weight		Pro- teins	Lipoids	Extrac- tives			
		<i>days</i>	<i>grams</i>	<i>grams</i>							
1	Normal ¹	2021	351	2.001	78.4	47.4	39.4	13.2	8.1	22.3	9.9
2	Ataxic	600	309	1.799	79.3	49.2	36.8	14.0	7.7	20.8	8.0
3	Normal	598	334	1.903	79.3	48.8	37.4	13.8	7.8	22.4	8.0
4	Ataxic	206	354	1.852	79.8	50.2	36.8	13.0	7.8	22.7	9.1
5	Normal	205	297	1.888	80.2	50.6	36.0	13.4	7.8	23.1	9.8
6	Normal	183	362	1.879	79.8	50.7	37.1	12.2	7.5	23.5	6.3
7	Ataxic	166	314	1.784	79.5	49.7	37.2	13.1	7.4	23.0	6.1
8	Ataxic	158	326	1.789	80.2	52.1	34.9	12.9	6.8	21.9	6.3
9	Ataxic	133	331	1.900	79.6	50.9	36.4	12.7	7.1	22.4	(8.1)
10	Normal	106	360	1.922	80.0	50.0	36.8	13.2	7.0	22.9	6.5
11	Mixed ²	45	217	1.475	82.6	51.9	33.7	14.4	6.5	22.8	4.1

¹ Birds not of ataxic strain, but of nearly similar variety.

² A mixed group, probably normals and ataxics, all from ataxic strain.

NOTE.—Nos. 1 to 5 and 11 are new data; nos. 6 to 10 are our earlier data ('18).

is low in both; extractive-phosphorus and protein-phosphorus are high in at least one case. In all of these fractions these two ataxic brain groups are less differentiated chemically than brains of their calendar age should be. Extractives are not distinctive of age for the ages actually considered and one ataxic shows a high the other a low figure for this fraction.

It thus appears that of those nine chemical fractions (eighteen for the two groups) which can be relied upon to reflect age differ-

¹⁴ Confirmed by lipid-phosphorus and lipid-sulphur, table 7.

ences in the whole brain of the two most strongly affected groups, two or three fractions indicate equivalent age, two indicate older age, and thirteen fractions indicate younger age than was actually theirs. It is difficult to believe that such results would have been obtained on two groups of brains not actually unlike in degree of chemical differentiation. Similar differences in smaller degree and of less definiteness occur in the one strongly ataxic cerebrum and cerebellum-medulla group analyzed. The whole brains of two additional groups of birds showing relatively little ataxia gave nearly indifferent figures in respect to age. It seems necessary to conclude that the result of the two series of brain analyses indicates that chemical differentiation does not proceed as rapidly in the brain, perhaps more particularly in the cerebellum-medulla of ataxic birds as in the brain of normal birds. Moreover, chemical under-differentiation of the ataxic brain certainly may persist into very mature age.

DISCUSSION

Analyses and materials

In our analyses we have been obliged to deal with groups of brains and not with individual brains. This fact has a bearing on the results obtained. The ages of some of the birds of an older group were not very dissimilar to that of some of the birds placed in a younger group. The material entering into the samples is further complicated by the possibility that some among the birds considered as normal might later have shown obvious ataxia. The ataxia manifests itself in various degrees and becomes evident at various ages. Ataxia exhibited in early life may later wholly disappear. Some of the ataxics selected may have been well under way to recovery. It seems probable that the observed differences in chemical composition between ataxic and normal brains would have been greater if it had been possible to analyze single brains instead of groups of brains. In connection with these remarks, we would ask that it be borne in mind that unlimited numbers of ataxic birds have not been available to us, since the derangement, though hereditary, behaves rather as a recessive than as a dominant (Riddle, '18).

Our data concerning the localization of the derangement in the brain are still imperfect, because in our analyses the brain was separated into anterior and posterior parts only. It has been made clear that the chief size reductions occur in the posterior brain; and the evidence indicates that the deviations in chemical composition are accentuated in this same region.

Whether analyses of medulla and cerebellum separated from each other would have shown that all of the size and chemical changes occurred in one only of these organs is a question quite unanswered by our data. Nevertheless, the fact that changes were also found in the cerebrum would seem to indicate that the derangement is not absolutely confined to either of the chief divisions of the brain. It is possible, however, that localized affected areas are present and that these were 'diluted' by much normal material in our samples as prepared for analysis. If this were true, these particular localized areas would necessarily have a much greater degree of chemical under-differentiation than is indicated by the figures obtained by us.

The sex of the ataxic birds deserves a further statement. Those who may have carefully examined the character of the samples obtained from ataxic pigeons, in both the earlier and present series, will have noted that more female brains than male brains are found in these samples as prepared for analysis. In the earlier series (of ataxics) the proportion was ten females to five males; in the present series twelve females to eight males. This disproportionate representation of the two sexes in these samples was not consciously effected by us, since the sex of most of the individuals selected for the purpose was not known until after the birds were killed. In most cases they were selected chiefly because they were ataxic in one or another degree. Equality of the sexes was desired in our present samples, but could not always be obtained.

The excess of females in the two series of ataxics has led us to examine a segment of the breeding data in an effort to learn whether the ataxia more often occurs in females than in males. The data given below were obtained from a tabulation¹⁵ of the

¹⁵ All groups of offspring of ataxic blood or strain were included in the summary. The matings which had yielded no ataxic offspring were excluded.

offspring (to the fourth generation) of the original ataxic female. These data indicate that the ataxia does occur more frequently among females if the computation be made upon offspring which live long enough to permit a reasonably accurate prognosis of ataxia or normality. Obviously, no other method of computation is practicable.

Necessary to a full consideration of these particular figures are the facts, published earlier by one of us (Riddle, '18), that the ataxia does not behave as a sex-limited character in heredity, and that probably more affected than unaffected individuals die early—before definite classification as ataxic or normal is possible. Also, fully as many females as males die early. Included in the group of birds that were properly classified for the above purpose are a total of seventy-seven males and seventy-five females. Among these there were, however, only fifteen ataxic males to twenty-nine ataxic females. It is therefore quite probable that females are more subject to the derangement than are males.

Comparison of constituents of parts of human and pigeon brain

As a result of our separate analysis of anterior and posterior parts of the brain, it is now possible to make a comparison of these with similar parts of the human brain. So far as we are aware, there are no data for other animals which permit a similar comparison with the parts of the human brain. The comparison is best made by reference to table 9. A study of the figures obtained brings to light the rather surprising situation stated below.

The human cerebrum has, of course, higher values for certain chemical components and lower values for certain other components than the human cerebellum-medulla. The same is true for the corresponding parts of the pigeon's brain. The singular fact to which attention is directed lies in the circumstance that in the pigeon the direction of the difference is the reverse of that for man in the case of every chemical fraction shown in the table.

Perhaps this incongruity will not be made less intelligible by the immediate statement of another peculiarity of the figures of

this table. These show that (from the standpoint of relative amounts of the various chemical constituents) the cerebellum-medulla of the pigeon is chemically an intermediate of the pigeon cerebrum and the human brain (both of cerebrum and of cerebellum-medulla). Only the sulphatids of the seven fractions from the pigeon cerebellum-medulla fail to take an intermediate place between the pigeon cerebrum and human cerebrum.

TABLE 9
Comparison of the chemical composition of the adult cerebrum and cerebellum-medulla of man and of the pigeon (in per cent of solids)

	WATER IN PER CENT	PRO- TEINS	EX- TRAC- TIVES	LIPIDS	PHOS- PHA- TIDS	CHOLE- STEROL	SUL- PHA- TIDS	CERE- BROS- IDES
(Part 1)								
Cerebrum								
Human ¹	76.9	37.7	7.9	54.4	28.3	10.0	9.6	6.6
Pigeon ²	80.3	51.0	13.9	35.1	22.4	7.5	7.6	— ³
Cerebellum-medulla								
Human ¹	78.1	40.4	8.7	50.9	25.0	6.4	9.0	7.4
Pigeon ²	77.9	46.1	12.6	41.3	23.5	8.9	12.5	— ³
(Part 2)								
Rearrangement of above figures in terms of decreasing (ontogenetic) age								
Human cerebrum.....	76.9	37.7	7.9	54.4	28.3	10.0	9.6	6.6
Human cerebellum.....	78.1	40.4	8.7	50.9	25.0	6.4	9.0	7.4
Pigeon cerebellum.....	77.9	46.1	12.6	41.3	23.5	8.9	12.5	— ³
Pigeon cerebrum.....	80.3	51.0	13.9	35.1	22.4	7.5	7.6	— ³

¹ Average of two analyses by Koch and Voegtlin ('16) of cerebrum and cerebellum-medulla.

² Average of two analyses (I-Ia and II-IIa of this paper).

³ Cerebrosides have not been determined in the pigeon brain.

If, now, the figures found in part 1 of table 9 be arranged in such an 'age series' as was prepared for the several pigeon brains of various ages (table 8), the result may be seen in part 2 of table 9. According to the places taken by cerebrum and cerebellum-medulla of man and the pigeon in this arrangement, the human cerebrum would seem to be the oldest—i.e., the most fully differentiated 'brain tissue;' the human cerebellum-medulla next in order; the pigeon cerebellum-medulla next. The pigeon cere-

brum would seem to be the least differentiated, or youngest, of these 'brain tissues.' Not quite all of the figures agree in the assignment of a particular brain type to its place in the series. Certainly, however, there is an interesting agreement.

Do these figures have any phylogenetic meaning? Does the known sequence of chemical differentiation in ontogeny have any relation to phylogenetic facts? As the types of brains stand in the series, the human cerebrum shows the highest chemical differentiation; the pigeon cerebrum is the least differentiated. The cerebella occupy intermediate positions, that of the pigeon being lower than the human.¹⁶

Of course, differences in proportion of white and gray substance are involved, but possibly neurologists may have at hand, or may later note, other facts which have a relation to these comparisons of chemical composition and to this grouping of chemical types of brain on the basis of age. It has already been shown by Donaldson ('08, '10) that two main phases of brain growth in man and the rat are similar at corresponding ages and that the percentage of water in the brain agrees at equivalent ages. Hatai ('17) concluded "that the percentage of water (in body of different mammals) is an indicator of the chemical alteration in different species, while neither the calendar age nor body weight of the animals can be used for this purpose."

¹⁶ It should be shown that the relative position of these brain parts is not a fortuitous result of the particular ages of the human and pigeon brains selected for comparison. The human brains were aged 20 and 54 years; the pigeons were of 205 and 598 days. These pigeons are sexually mature at 180 days. Five hundred and ninety-eight days is more than three times the period preceding sexual maturity. By this method of computing age, the two groups seem comparable. If, moreover, the figures for either the 205-day or 598-day birds be taken to represent properly the composition of the pigeon brain, none of the figures of the two parts of table 9 are changed or misplaced in relation to the other figures. If the 20-year human alone be made to serve as a basis of comparison, the order of none of the figures is changed. If the 54-year human be made to represent the human, then the only changes of order concern the moisture of the human cerebellum-medulla which falls slightly below (initially it is only 0.2 per cent above) that of the pigeon cerebellum-medulla, and thus makes the series more perfect for the water fraction than it stands in the table. One slight additional change results: The extractives of the human cerebellum-medulla fall very slightly below the extractives of the human cerebrum.

The 'age series' of pigeon brains

It has earlier been stated that it is only on the brains of man and the rat that we have had fairly adequate data for the progressive change of the various proximate chemical constituents during growth, or, more properly, as related to growth and age. The present work supplies such an 'age series' for the pigeon brain, and this series is now as extensive as are those now known for man and the rat. Each of these latter series includes observations on one or more relatively younger stages than we have studied in the pigeon. On the other hand, the data for the pigeon include one relatively older stage than has been obtained on either of the other two forms.

Except for differences which appear because of a lack of parallelism of age, the three 'age series' show that quite the same course of chemical differentiation is followed in the brain of man, the rat, and the pigeon. It is not our purpose to discuss these three series here. The essential similarity of results obtained on material from sources so unlike should, however, be noted as additional evidence for the trustworthiness of the methods developed by W. Koch ('09) for brain analysis. The brains of the three 'age series' mentioned above have all been analyzed according to Koch's method.

Since the above was written, we have had an opportunity to learn something of the results of the neurological studies made by Hoshino ('19) of the brains of some of this same family of ataxic pigeons. Although the present study was completed and fully described before we were aware of Hoshino's results,¹⁷ it seems well to add here that the neurological and chemical studies support an essentially similar view. The bearing of Hoshino's summary statement is self-explanatory: "This may be regarded as a hypoplasia or developmental inhibition in the proprioceptive system, part of the motor system, and some structures connecting the medulla oblongata and cerebellum, occurring during growth, with scarcely any definite degeneration or secondary increase of neuroglia tissue."

¹⁷ The courtesy of Doctor Hoshino has made it possible for us to read his completed manuscript prior to its publication.

SUMMARY

1. The brains of birds which have lost a very large amount of the normal control of the voluntary movements (ataxia) show deviations from the normal brain in size and in chemical composition. These deviations are more pronounced in the cerebellum-medulla.

2. The brains of the ataxics are smaller. The cerebrum is either not reduced or is reduced in very small amount. The cerebellum-medulla (weighed together) is certainly reduced in size.

3. Possibly the somewhat smaller brain size of the (mature) ataxics is necessarily associated with a relatively less amount of white substance. If this is true, some, but not all, of the observed inequalities in chemical composition may be associated with this circumstance. The whole of the results would nevertheless emphasize the existence of some retarding influence on the completion of growth in the ataxic brain.

4. Eight analyses were made of anterior and posterior parts of the brain. Four of these were from ataxic birds and four from normal birds. The chemical changes found are more definite and pronounced in the cerebellum-medulla than in the cerebrum. The results support our previous conclusion that the differences "suggest a chemical under-differentiation or immaturity of the ataxic brains."

5. The pigeon cerebrum and cerebellum-medulla strongly contrast with the human cerebrum and cerebellum-medulla in the distribution of the several chemical constituents.

6. Entire brains of very young and of very old birds were analyzed. Data for the chemical changes in the brain which accompany age have been obtained for a series of ages in the pigeon. Examination of this more extensive 'age series' of pigeon brains has enabled us to evaluate much better than in our previous work the relation borne by the various chemical fractions to age and has also drawn attention to the relatively greater absolute brain weight in the males and the relatively greater weight of the cerebellum-medulla as compared with the cerebrum in the females.

7. The significance of the results obtained in the present and former series of analyses has been reviewed. The evidence warrants the conclusion that chemical differentiation, probably represented largely by the relative abundance of the myelin, does not proceed as rapidly in the brain, and more particularly in the cerebellum-medulla, of ataxic birds as in the brain of normal birds.

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Resumen por el autor, Teiji Hoshino.

Estudio del cerebro y médula espinal de una familia de palomas atáxicas.

El autor ha estudiado funcional y anatómicamente cuatro palomas que presentaban ataxia hereditaria y tres palomas normales de la misma familia, las cuales le fueron enviadas por la Estación de Evolución Experimental de la Institución Carnegie. Incluye en el presente trabajo la historia completa de la familia aludida con los datos de anatomía gruesa y microscópica referentes a los individuos atáxicos y a los normales que sirvieron como tipo de comparación. Los cambios encontrados en el sistema nervioso central consisten principalmente en una reducción del tamaño del cerebro y médula espinal, especialmente en el cerebelo y las partes directamente relacionadas con él. Esto puede considerarse como una hipoplasia o inhibición del desarrollo del sistema propioceptivo, parte del sistema motor y algunas de las estructuras que unen a la médula oblonga con el cerebelo, la cual tiene lugar durante el crecimiento, con una degeneración apenas marcada o aumento secundario del tejido neuróglíco. Después de revisar someramente la ataxia de Friedrich y la hérédito-ataxia cérébelleuse de Marie, el autor interpreta la condición de las aves examinadas como una combinación de las dos afecciones humanas mencionadas.

Translation by José F. Nonidez
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A STUDY OF BRAINS AND SPINAL CORDS IN A FAMILY OF ATAXIC PIGEONS

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THREE FIGURES

Although it is said that hereditary disturbances of coördination in man are very rare, still not a few reports have been published, especially since Friedreich ('63, '75) and Marie ('93) described the disturbances from both an anatomical and clinical point of view. Of similar conditions in lower mammals, three cases have been reported: in the kitten by Krohn ('92), Langelaan ('07), and Jelgersma ('18).

So far as I could find no authors have hitherto investigated hereditary incoördination in birds. Such a study might throw some light on the comparative and pathological anatomy of this condition.

The birds which form the foundation of this report were presented by Dr. Oscar Riddle, of the Carnegie Station for Experimental Evolution, Cold Spring Harbor, Long Island, New York, to Dr. C. J. Herrick, Professor of Neurology in the University of Chicago, who was good enough to turn them over to me to study the changes in the central nervous system. I am indeed very much indebted to these gentlemen for giving me such an opportunity, and in particular to the latter who has given me valuable suggestions in the course of the investigation. I also wish to thank the members of the anatomical department who so kindly made it convenient for me to carry on this work.

Doctor Riddle has sent us a very exact and complete family history of the birds. He has studied heredity in pigeons for several years, continuing the work of Professor Whitman. The history will be interesting, for in man we seldom find such an exact and

reliable record of hereditary diseases over a period of several generations. Therefore, it will undoubtedly be interesting to copy from his notes and from one of his reports ('18) the remarkable parentage of these pigeons. The following is a brief summary of this history.

From an egg produced by the weakening influences of 'reproductive overwork' a female pigeon no. 151 was hatched in 1914 which showed a marked lack of power over the voluntary movements of the head and body. This lack of coördination was practically completely lost in the adult bird. The affected female was bred to two normal males, A126 scraggly and C-B9. The derangement has been inherited through four generations descended from either male.

The parents of no. 151 were raised by Professor Whitman. The male parent, a two-barred homer H-A, had no ataxic symptom nor did his sire or dam. H-A homer was an inbred, for its parents were brother and sister. The dam of no. 151 was of the homer-carrier type, normal and without ataxia. These parents of no. 151 laid for the last time in 1914 on about October 12th to 14th, and one of these eggs hatched the ataxic female. This female (no. 151) was thus hatched at the end of the season from a pair of birds which had been kept constantly at work and from parents one of which was an inbred.

When first out of the nest the abnormality of no. 151 was noted, and therefore the next pair of eggs produced by parents of no. 151 were also incubated. The two birds hatched from these eggs resembled no. 151, but were not ataxic. There is no record of ataxia in any of the other descendants of the parents of no. 151 during the entire previous four years. There is reason to believe that this character arose within the germ that produced no. 151 and that the weakening effects of abnormally rapid egg-laying and possibly the inbreeding of the male parent were causally related to the appearance of the character.

The sire (A428) of the 'scraggly' male no. A126 was a checkered *Columba livia domestica*, which had the tips of the wings white. As is well known, white is apt to appear in these outmost wing-feathers in many breeds of the domestic pigeons. This restricted

sort and placement of white seems to be the only trace of white that could be carried by either the 'scraggly' male or the 'ataxic' female. The dam (545) of no. A126 was not very accurately described for color, but was probably of medium slate color and two-barred. The possibility of some white primaries is not excluded, but is wholly improbable. Her sire was of slate color and two-barred; her dam was wholly black. Her brothers (of whom one was a 'scraggly,' from an 'alcoholized' egg) and sisters bear no record for white in any case. Her offspring, the several brothers and sisters of A126, ranged in color from light slate with two bars to black; no white appeared in any birds of this fraternity. The dam of 'scraggly' A126 also threw a 'scraggly' female (A339) from an 'etherized' egg. The dam, no. 545, was herself hatched from an 'alcoholized' egg, and from the eighteenth egg laid within a period of ninety-two days. No. A126 was produced out of season, February 1, from the tenth egg in life, these ten eggs being produced in the very short period of forty-seven days.

The above records for 'scragginess' in connection with the mother of 'scraggly' no. 126 would raise a question as to whether 'scragginess' were not carried by the mother, and thus did not originate in the germ that produced A126. This is a question that cannot, of course, be definitely settled. It is of importance to note, however, that the 'scragginess' in this fraternity is found only in 'treated' germs (alcohol, ether), or in the offspring (A126) of a bird from a treated germ, and also in all cases in connection with weakening influences of reproductive overwork. Scragginess had appeared earlier several times in birds of various strains in the long history of our collection of pigeons, but it had been observed that such birds arose more frequently or entirely from 'weakened germs'—of late season or out of season, and from parents 'worked' more rapidly than normal. To us it therefore seems more probable that the germ which gave rise to A126, if developed, grown, and liberated under wholly favorable conditions instead of the reverse, would probably have produced a normal bird; and if, then, in turn, the germs produced by this normal bird had been favored by the best and most normal conditions the character would probably not have been exhibited in its offspring.

Male C-B9, with which the ataxic female (151) was mated for a short period prior to her mating with the scraggy male, was a pure wild rock pigeon (*Columba livia*). It was hatched in 1910 from parents obtained (1908) from the caves of Cromarty, Scotland. The three offspring of this very strong and vigorous male and the ataxic female were normal in appearance and behavior; but in the next generation a portion of the offspring exhibited ataxia. No white color has thus far appeared in any of their descendants.

Ataxia, scragginess, and white color have all appeared in three generations derived from the mating of the scraggly male and ataxic female. Without here entering into full considerations of the proportions of abnormals to normals for each of these three characteristics in the different generations, it can be said that the first generation showed relatively few abnormals—ataxics, scragglies, or whites. Later generations have shown higher proportions of affected individuals, and the combination of ataxia and scragginess has there been obtained.

The ataxia of the original ataxic bird (no. 151) disappeared some time after she became adult. When she died recently, she seemed quite normal. This is not true of many or most of later ataxics, which show much more extensive lack of coördinations, and maintain them till the end of life. Of course, the extreme ataxics do not live long. Doctor Riddle describes the scragginess as follows: This, he says, is a plumage defect; the feathers lack barbules and hooklets, and as a result the barbs of all feathers of all these birds hang loosely apart so that the wing feathers give no resistance to the air, and the birds cannot fly. Such feathers present a very peculiar and bristling appearance.

The statement concerning pedigree, and behavior of each of the four birds, which were sent us runs as follows:

No. K137. Young of cage 131. Second generation hybrid (not counting original ataxic and scraggly as first generation). Parents: male A456 and female A446 (neither of which showed ataxia or scragginess). The parents of these latter: original ataxic female 151 and original scraggly male A126, from eggs laid 4/8/17. Ataxic—gait unsteady; flies very little or not at all; tips backward, and also tends to tip sidewise.

No. K172. Young of cage 269a. Third generation on one side; second generation on other. Side of third generation is through a normal brother of no. K131, described above. Side of second generation is through ataxic female no. B661, which is offspring of original ataxic female no. 151 mated to pure wild rock pigeon male C-B9. From egg of 5/19/17. Ataxic—tips backwards.

No. K158. Young of cage 130. Second generation from two normal young of the two last-named birds: ataxic 151 and normal C-B9. From egg of 6/17/17. Ataxic—tips or nods head sidewise; tips backward, and sometimes flies sidewise.

No. K207. Young of cage 123aa. Second generation, from two normals, male B533 and female B548; these latter were offspring of original ataxic female 151 and scraggly male A126. From egg of 6/22/17. Ataxic—somersaults backward, occasionally falling sidewise; twists neck and head; does not fly; no coördination in any movements observed.

No. K167. Normal, is of same fraternity as K137. From egg of 6/19/17.

No. K199. Normal, is of same fraternity as K207. From egg of 6/9/17.

No. B473. Normal, older bird, is of same fraternity as K158. From egg of 12/21/16.

With the above detailed records we received the four ataxic and three normal birds in good condition on October 26, 1917. We observed them for more than three months, during which time all of the four ataxic pigeons slowly became worse, while the three normal ones seemed quite healthy and in good condition, living a very active life.

The affected birds apparently are backward in their development, they look smaller, their feathers are scanty; they have lost the characteristic brilliant color, and appear lusterless. The muscles are flabby. The birds maintain one position quietly almost constantly. To support the body they stretch their legs wide apart and a little forward with the tail braced against the bottom of the cage and the trunk partly lowered to the floor, so as to avoid falling forward, backward or to the side. The pigeons, then, maintain their position while standing with three supports just like a three-legged stool; two widely spread legs and a tail braced against the floor. The affected birds do not stand on the limb of a tree as normal pigeons usually like to do, but remain on the floor of the cage, often supporting themselves

on one side of the body with the wall of the pen. If food or water is placed in the middle of the cage on the floor, they have great difficulty in reaching it. Food is really the only thing which will make them attempt to walk, except when they are frightened or excited. In their attempts to walk they fall forward or sideways or just stumble along reeling like a drunken man, '*démarche ébrieuse*.' When they fall forward they try to get up with their bills against the floor pushing back the body and flapping the wings with much effort. When they fall to the side they usually roll over once or twice. Sometimes they fall to the right, while at other times they fall to the left and then roll until they reach some obstruction which helps them to get up with the aid of flapping the wings. Flying is practically impossible in all birds; if they are thrown free in the air, they flap their wings irregularly and cannot fly above the height they are thrown, but go directly down to the floor notwithstanding that their flapping efforts are much more intense than those of normal birds. When the birds are excited or frightened, the disturbances of the irregular movements stated above are much more apparent. Such a movement as the so-called "tremulance" or oscillatory movements cannot be observed either when the birds are excited or at rest. Ocular movements are free, no deviation and no nystagmoid jerking can be substantiated. The reflexes which may be elicited from the cornea are normal. When they are put on a rotating chair they show the head nystagmus characteristic of normal pigeons. If they are rotated more than five or six times they lean against the cage wall or lie down, exhibiting regular head nystagmus. When blindfolded the birds reveal no increase of the disturbances of coördination.

As far as can be determined, sight and hearing are normal; the birds can recognize food and an observer who may be approaching; they also react to a sudden sound by raising the head and trunk suddenly, but immediately lower them again. Pupils are equal and react to light promptly. The sensibility to touch as well as to pain appears unaffected in the skin; the birds react to stimulation with direct movements, but all these movements are quite sluggish. The toes of three affected pigeons are more or

less flexed and widely abducted, so that the web spaces appear quite large and toes show so-called hollow-foot. They do not 'coo' or make any other noise. To observe the intelligence of a bird is of course always difficult. So far as we can see from the behavior of the birds, the intelligence seems not to be far different from that of the healthy birds; they show movements of uneasiness and fear, if one approaches them or tries to catch them then they begin to move away as one approaches. They distinguish food from uneatable objects, and show a preference for the place in the cage where the body is most conveniently supported.

No bird shows a limitation to a particular kind of incoördinated movement; all of them have a nodding head and neck and even a swaying trunk, tipping forward and backward and falling to either side. The unsteady staggering gait, tipping head, and swaying trunk are the common symptoms in all affected birds with of course variations in degree. A weakness of the sphincter of rectum or bladder cannot be detected in any bird.

Pigeon no. K207. Keeps body quiet; stretches the legs forward and widely apart laterally, the head and neck pulled a little backward. The bird tips to one side twelve to fifteen times when standing without leaning against the wall or on any support. When excited the head and neck move at first clonically upward, then backward so that the head eventually touches the back. To coördinate this forced position of head and neck the bird flaps the wings excitedly, but in vain, to restore the right position. Often the bird turns a somersault backward several times to regain its position. If we catch the bird by the wings, we feel a strong resistance in the wing muscles when the forced movements are occurring. No rigidity or paralysis, however, was recognized (fig. 1). The pigeon was killed January 28, 1918.

Pigeon no. K137. Remains on the floor with widely spreading legs and with tail braced against the bottom of the cage. When walking the bird tips to one side or the other. When excited this movement occurs twelve times a minute. Usually always when two or three steps are taken the bird stops and braces the tail against the floor to regain its equilibrium. Reacts normally to light and sound. No spontaneous nystagmus in head or eyes. As time progressed the swayings of the body increased so that before it was killed (February 2, 1918) they occurred eighteen times a minute. The legs react to touch and pain, though slowly. The nails and phalanges of the toes were flexed and the toes turned toward the midline of the body resulting in a sort of talipes cavus or hollow foot. The tail, owing to constant use as a

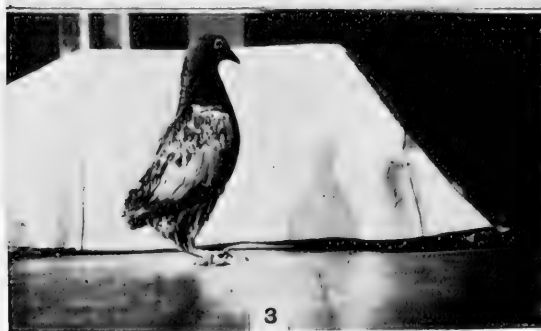


Fig. 1 Pigeon K207. Most ataxic, the head and neck twisted and turned backward, the lower part of the body braced on the floor, legs stretched laterally and forward, phalanges of toes flexed resulting in hollow foot.

Fig. 2 Pigeon K137. Body swaying forward with flapping wings. The wings and tail are shortened.

Fig. 3 Pigeon K172. The tail and wings are short, owing to frequent use as supports of the trunk; the feathers are worn down and appear as if they had been cut off with scissors.

support of the trunk, is short and the feathers are worn down, and appear as if they had been cut off with a scissors (fig. 2).

Pigeon no. K172. Keeps quiet and still as if sleeping, swaying only the head and neck toward the side and a little backward. The bird assumes the same position as the two previous ones. A wink of eyes is often observed. No spontaneous nystagmus in head or in eyes can be seen. The bird reacts to sounds. The most lateral of the three front toes of the right foot is bent backward rather than forward. The tail is shortened and has only rough feathers. This bird cannot fly at all. When excited the bird rises up and a little to the side and then bends backward slowly in clonic contraction till the forehead touches the back; with flapping wings a somersault is made backward or the bird falls to the side. From December 18th, the pigeon could not maintain the body in a standing position, but fell every five seconds to the side and backward. When it fell on the back it could hardly restore its normal position. On January 14, 1918, the bird was killed (fig. 3).

Pigeon no. K158. Is the most slightly affected one. Owing to the tipping or swaying of the head and neck toward the side or forward, the rapid coöordinated movements of feet and legs forward or to side can be observed. Sometimes to maintain equilibrium in these irregular movements the bird flaps the wings. These movements occur about twelve times a minute, but the movements are slight and the restoration of the body position occurs quickly. During the whole period of observation, it never tipped or swayed backward. Bird walks swaying from side to side just as is done on board ship in a rough sea. It will not fly alone, but if set free in the air, it will fly to a higher level than the position where it is set free. At rest the body is supported on forward stretching legs with tail on the floor. Only a slight deformity of the toes on both sides is seen. Killed February 18, 1918.

As for the three healthy birds, they never revealed any abnormality of movement during more than three months of observation, but lived a lively life, cooing, flying, or else perching on the limb of the small tree in the cage.

METHODS OF PREPARATION

In reference to the examination of the central nervous system of the birds, we must remember that the anatomy of the normal tracts and nuclei of the pigeon is yet much in the dark, notwithstanding the works of Stieda ('69), Turner ('91), Brandis ('93-'96), Friedlaender ('98), Wallenberg ('98-'06), Edinger ('03-'08), Ramón y Cajal ('08), Frenkel ('09), Kühn and Trendelenberg ('11), Shimazono ('12), Ingvar ('18), and others (Kreis,

Winkler, Dogiel, Münzer and Wiener, Boyce and Warrington, Murphy, Ziehen, Williams and Brouwer). It is not easy, therefore, for any one to study accurately any changes that have occurred in the nervous system of the pigeon. For this reason each section of the affected birds was treated quite the same way as a corresponding control section. This not only gives us a comparison with the normal structure, but also serves to show us any artefacts that may be present.

The birds were narcotized with ether, while I opened the cranial cavity and spinal canal to take out the whole brain and spinal cord. During the time of the removal the brain and cord were both rinsed in physiological salt solution, and before fixation, measured and weighed. The brain stem, cerebellum, and the spinal cord of both the normal and affected birds were used for microscopic examination. The brain was cut through proximally at the level of the posterior third of the optic lobes and distally at a point separating the medulla oblongata from the spinal cord. Half of the cerebellum was left attached to the medulla and the whole fixed in 10 per cent neutral formalin solution, while one portion of the half of the cerebellum removed was fixed in Zenker's formalin solution and the other in alcohol. The following is the formula for Zenker's formalin solution used:

Bichromate of potassium.....	2.5 grams
Bichromate of mercury.....	.5 grams
Sodium sulphate.....	1 gram
Formalin.....	10 cc.
Distilled water.....	100 cc.

From the spinal cord two parts were taken, one from the cervical region and the other from the lumbar region. Each piece was cut into three divisions, one put in Zenker's formalin, one in 10 per cent neutral formalin, and the third in alcohol. Each specimen had a control piece from the normal bird and both were treated the same way in the same bottle. After fixation, regular dehydration followed with both pathological and control specimens. Both pieces were imbedded in the same block of paraffin and cut with the microtome at the same time. With each stroke of the blade, then, two sections would be made, one pathological

and the other normal, and both having the same thickness. The two corresponding sections were then put on the same slide and stained at the same time. This does away with any variation that could arise from the dye. In reference to the stains, Weigert and Pal's modification, toluidin-blue-erythrosin, Mann's eosin-methyl-blue, haematoxylin-eosin, and Mallory's neuroglia method were employed.

Thus, each affected section and normal control section has been treated in exactly the same way from fixation to cutting and staining, and we are able to compare any slight pathological changes which might have taken place with much reliability. If there are any artefacts or postmortem changes, which must occur to some extent, they will be present in both the affected and normal specimen to the same degree. The findings observed in each of the sections of all the birds will be here recorded together, for the changes found are almost the same in all four affected pigeons.

MACROSCOPICAL FINDINGS

The small size of the brain and spinal cord in all affected birds can be recognized at a glance without any hesitation, especially in the spinal cord, cerebellum, and medulla oblongata. The length of the spinal cord and the weight of the different parts of the brain and cord may be seen in tables 1 and 2.

As is seen in the tables, the weights of the brain and spinal cord are not only absolutely less in the affected pigeons, but less in proportion to their body-weights. In the affected pigeons, moreover, there is much more reduction in weight of the distal portion of the brain which includes the cerebellum and medulla oblongata chiefly, but also a part of the midbrain and the lower third of the optic lobe than of the proximal part of the brain which is the cerebrum chiefly. Again, the ratio between the weight of the distal portion of the brain to the whole brain in the affected pigeon is always less than the ratio between the weight of the distal portion of the brain to the whole brain in the normal pigeon. The same relation holds for the spinal cord.

TABLE 1
Weight of brain and spinal cord

	K137 (A)	K167 (N)	K158 (A)	B473 (N)	K207 (A)	K169 (N)	K172 (A)	AVERAGE NORMAL	AVERAGE AFFECTED
Sex.....	♂	♂	♀	♂	♀	♂	♂		
Age (days).....	300	228	246	424	220	232	230	293	248
Body weight (grams).....	343	371	315	358	318	364	339	364	329
Weight of whole brain.....	1.833	2.004	1.699	1.987	1.686	1.941	1.840	1.977	1.763
Weight of brain and spinal cord.....	2.515	2.801	2.289	2.722	2.342	2.752	2.528	2.758	2.419
Weight of spinal cord.....	0.682	0.797	0.590	0.735	0.656	0.811	0.688	0.781	0.654
Weight of the proximal portion of brain ¹	1.220	1.262	1.158	1.295	1.155	1.222	1.270	1.257	1.201
Weight of the distal portion of brain ¹	0.613	0.742	0.541	0.692	0.535	0.719	0.589	0.718	0.570
Ratio weight of brain and cord to body weight.....	1:133.3	1:132.4	1:137.6	1:131.5	1:135.7	1:132.3	1:134.0	1:132.0	1:135.9
Ratio weight of the distal portion of brain to whole brain.....	1:2.990	1:2.687	1:3.142	1:2.871	1:3.153	1:2.699	1:3.123	1:2.769	1:3.098

¹ See 'Methods of preparations' and 'Macroscopical findings.'

The spinal cord is shortened in the affected subjects. None of the cases presents either a scoliosis or kyphosis of the vertebrae. The medulla oblongata is reduced in size in both the ventrodorsal and transverse diameters. Nowhere in the brain or spinal cord can there be observed a defect of any region with the naked eye or with a lens. The cerebellum viewed from a cut surface in sagittal section, after fixation, exhibits a rather round outline in the affected specimen, while in the normal the anterior, dorsal, and posterior margins are easily distinguishable, the whole ap-

TABLE 2
Length of spinal cord

	K137 (A)	K167 (N)	K158 (A)	B473 (N)	K207 (A)	K199 (N)	K172 (A)	AVERAGE NORMAL	AVERAGE AFFECTED
Length of the whole spinal cord in millimeters.....	163	173	160	171	151	178	169	174	161
Length from the upper part of the cervical cord to the beginning of the upper intumescent a.....	59	63	57	62	55	63	60	62.7	57.8
Length from the upper part of the cervical cord to the beginning of the lower intumescencia.....	120	129	118	127	111	131	125	129	118

pearing as a five-angled polygon. All the lobuli and sulci of the cerebellum are sharp and well defined in the normal specimens, while in the affected specimens the lobuli are thin and flat and the sulci shallow, the whole appearing much more indistinct. In each, however, the total number of lobuli is the same.

The consistency of the brain substance is the same in both the affected and normal birds; one can feel no sclerotic hardness in the affected brains.

The visceral organs in all affected birds reveal no abnormal conditions except that the testes in pigeon no. K172 are somewhat rudimentary (one-third of normal size).

MICROSCOPICAL FINDINGS

1. *Spinal cord*

The spinal cord of the pigeon has two enlargements, the upper and lower intumescencia. The upper enlargement is located far posteriorly, owing to the bird's long neck, and hence there is a very short thoracic cord between the upper and lower enlargements (cervical vertebrae, 14, thoracic 4, lumbosacral 7 or 8, coccygeal 5). The upper enlargement has a larger diameter than any other part of the cord. At the lower enlargement, the cord is divided into two halves by the 'sinus rhomboidalis,' as named by Kölliker ('02). The two halves of the cord are connected at the lower part of the intumescencia only by the anterior white commissure. According to Kölliker, this sinus is formed by extensive development of the sulcus dorsalis medialis in which there is a gelatinous glial tissue. The ligamentum denticulatum, a band of connective tissue which supports the cord from the lateral edges of the vertebral bodies, appears at the level of the lower enlargement well developed in the anterolateral portion of the cord.

White matter. All the sections of the spinal cords at the different levels in the four affected pigeons are decidedly small in reference to both the white and gray matter as seen with the microscope as well from the exact measurements, compared with the sections from the corresponding levels of the normal control birds. The myelin sheaths stained by the Pal-Weigert are generally slightly paler, so that each section of the affected specimens looks as if it were cut much thinner than the normal section, whereas, in fact, they are both exactly the same in thickness, as already indicated. Nevertheless, there is not found any area in the funiculi totally without color by Pal's method.

Throughout all levels of the spinal cord there is a relatively pale area in the median portion of the anterior funiculus and in the dorsolateral periphery of the lateral funiculus, while on the other hand the whole dorsal funiculus is pale. The other portions of the different funiculi do not exhibit any marked color change.

The one in the medial portion of the funiculus ventralis is shaped like a right triangle with the right angle in the corner between the edge of the cord and ventral sulcus. The triangle is elongated ventrodorsally and narrow from side to side in the upper cervical region, but it is broad from side to side and narrow ventrodorsally in the lower enlargement. This area is present in both normal and affected specimens, but in the affected ones the boundaries are quite indistinct. The normal has fibers of almost uniform caliber in this area, but in the affected one the fibers are small on the average and vary in size (tables 3 and 4). In addition to this, there are many small fibers under $2.8\ \mu$ in caliber in the affected birds. So many small fibers in this area are not observed in the normal preparations. They have all about the same size and their myelin sheaths stain deeply. These differences of the myelin sheaths and the variable caliber of fibers of the affected specimens no doubt give rise to the pale appearance and to indistinct boundaries of this area. The greatest transverse breadth of the funiculus anterior is reduced in all the affected specimens. Nowhere is there any apparent sign of the degeneration of the fibers, however. At the lateral portion of the anterior funiculus of pigeon no. K172, just at the place where the anterior rootlets pass through the white matter, from the ventral horn, the longitudinal fibers are arranged loosely.

The second area at the lateral periphery of the lateral funiculus, just dorsal to the dorsal horn, is long and crescent-shaped with its base toward the periphery. At its median side, it is bounded by an area of fibers of large caliber. This portion in the affected specimens is reduced in both the transverse and ventrodorsal diameters and is light blue in color; the myelin sheaths are thinner and the caliber of fibers is much smaller than normal, as is seen in tables 3 and 4. In the lower enlargement the fibers measure 2.9 to $7.1\ \mu$ in the normal and in the affected specimen 2.2 to $5.1\ \mu$.

The third area indicated as being the funiculus dorsalis, in addition to its pale color, has a brownish to red color when counter-stained by erythrosin, instead of the deep blue-black of the normal section. It must be noticed here that usually the funic-

ulus posterior in the normal pigeon stains a deeper blue to purple-black by Pal-Weigert than any of the other funiculi. In the affected specimens the median part of the funiculus posterior contains fibers of smaller caliber than normal and the fibers have poorly developed myelin sheaths though even the normal fibers

TABLE 3¹
The upper cervical region of the spinal cord

	K207 (A)	K199 (N)	K137 (A)	K167 (N)	K158 (A)	B473 (N)	K172 (A)	AVER- AGE NOR- MAL	AVER- AGE AFFEC- TED
Transverse diameter of cord in millimeters.....	2.738	3.340	2.137	2.204	1.970	3.206	1.870	2.595	2.097
Ventrodorsal diameter.....	1.903	2.171	1.753	1.887	1.118	2.004	1.720	2.020	1.745
Greatest breadth of ventral horn.....	0.217	0.283	0.250	0.350	0.317	0.334	0.217	0.322	0.250
Distance from the central canal to the latero-anterior periphery of the ventral horn.....	0.417	0.417	0.450	0.534	0.467	0.534	0.450	0.495	0.442
Greatest breadth of the dor- sal horn.....	0.133	0.183	0.233	0.301	0.233	0.300	0.150	0.272	0.188
Distance from the dorsal periphery of the dorsal horn to its base.....	0.300	0.300	0.384	0.417	0.417	0.434	0.384	0.384	0.370
Greatest breadth of the funiculus ventralis.....	0.417	0.450	0.450	0.534	0.501	0.584	0.501	0.522	0.467
Greatest breadth of the funiculus dorsalis.....	0.250	0.350	0.384	0.417	0.334	0.417	0.300	0.395	0.317
1) Number, and 2) size of large ganglion cells in { the anterior portion of { the ventral horn (μ)... {	8 19.9	16 37.1	3.5 25.7	8.5 28.5	4.5 28.5	11 42.7	6 28.5	11.8 36.2	5.5 25.6
Caliber of the fibers at the medial portion of the funic- ulus anterior (μ).....	7.9	11.9	8.5	11.4	8.5	10.2	7.2	11.1	6.1
Caliber of the fibers at the dorsolateral portion of the funiculus lateralis (μ).....	5.7	8.5	2.2	7.1	4.2	6.8	5.5	7.5	4.4
Caliber of the fibers of { the funiculus dorsalis { (μ) 1) medial portion; 2 2) Lateral portion..... {	1.9 2.8	2.8 3.5	1.7 2.2	2.6 2.8	1.9 2.8	2.7 3.4	2.4 3.1	2.8 3.2	2.0 2.7

¹The numbers given in each column in Tables 3 and 4 indicate the average obtained from twenty sections.

TABLE 4¹*The upper enlargement*

		K207 (A)	K199 (N)	K137 (A)	K167 (N)	K158 (A)	B473 (N)	K172 (A)	AVER- AGE NOR- MAL	AVER- AGE AFFEC- TED
Transverse diameter in millimeters.....		2.839	3.173	2.571	3.106	2.839	3.106	3.206	3.129	2.859
Ventrodorsal diameter.....		2.321	2.338	2.120	2.120	2.521	2.338	2.321	2.398	2.274
Greatest breadth of the ventral horn.....		0.668	0.801	0.551	0.701	0.668	0.734	0.734	0.746	0.654
Distance from the central canal to the latero-anterior periphery of the ventral horn.....		1.052	1.302	1.068	1.169	1.002	1.169	1.052	1.214	1.043
Greatest breadth of the dorsal horn.....		0.367	0.400	0.283	0.417	0.233	0.350	0.350	0.389	0.300
Distance from the dorsal periphery of the dorsal horn to its base.....		0.835	0.801	0.851	0.751	0.751	0.784	0.751	0.779	0.798
Greatest breadth of the funiculus ventralis.....		1.002	1.252	1.068	1.118	1.169	1.503	1.252	1.290	1.122
Greatest breadth of the funiculus dorsalis.....		0.384	0.501	0.384	0.584	0.584	0.751	0.584	0.612	0.486
1) Number, and 2) size of the large ganglion cells in the latero-anterior portion of the ventral horn (μ).....	1	15	24	12	28	18	32	26	28	17.7
	2	31.3	48.4	29.9	44.1	32.7	45.6	31.3	46.0	31.3
Size of Clarke's column:										
1) Broad diameter; 2) narrow diameter.....	1	0.384	0.384	0.301	0.367	0.251	0.401	0.284	0.384	0.271
	2	0.217	0.334	0.200	0.334	0.200	0.334	0.234	0.334	0.214
1) Number, and 2) size of the cells in Clarke's column (μ).....	1	7	21	7	15	4.8	17	4.5	17.66	5.56
	2	29.9	40.1	24.2	30.2	24.2	34.2	19.9	34.8	18.6
Caliber of the fibers at the medial portion of the funiculus anterior (μ).....		9.1	9.9	6.5	7.8	5.7	9.9	8.0	9.3	7.3
Caliber of the fibers at the dorsolateral portion of the funiculus lateralis (μ).....		2.8	3.4	3.1	5.7	2.2	3.4	2.5	4.1	2.8
Caliber of the fibers of the funiculus dorsalis (μ).....										
1) Medial portion	1	1.7	2.2	1.9	3.1	2.4	3.5	1.6	2.9	1.9
2) Lateral portion.....	2	3.4	7.1	3.4	6.8	3.6	5.7	4.0	6.5	3.7

¹The numbers given in each column in Tables 3 and 4 indicate the average obtained from twenty sections.

here are small in caliber, yet the difference between the affected and normal is well defined. The lateral area of the funiculus has a slightly larger caliber of fibers than the median portion, but even so they are smaller than those in the normal. In the upper enlargement, the medial triangular area in this funiculus along the median sulcus stains a deep blue to black in the normal, while in the affected one it shows a paler color and reduced breadth. The measurements of fibers are given in table 3. In the lower enlargement, the funiculi posteriores are not attached to each other, but they are separated by a wide space, the sinus rhomboidalis; the sinus side of each funiculus is convex, while the other side is closely applied to the dorsal horn. The funiculi in the affected specimens are not reduced ventrodorsally, but do show a diminution transversely. There appear to be two kinds of fibers in the funiculus in the normal pigeon. One occupies the medial fourth and measures $3.1\ \mu$ on the average, while the other group occupies the lateral three-fourths and has fibers of much larger caliber, $5.1\ \mu$. In these two areas of fiber groups, in the affected specimens, the fibers have a smaller caliber and thinner myelin sheaths; the average caliber of the fibers in the medial portion is $2.2\ \mu$, while those in lateral portion measure $4.2\ \mu$. Moreover, the arrangement of the fibers is looser.

No segmentation or decoloration in the myelin sheaths of the nerve fibers is seen.

Gray matter. The gray matter of the spinal cord of the affected birds shows a reduction in both the anterior and posterior horns and in the central gray matter. Both horns are reduced especially in width, as shown in the tables (3 and 4), but there is not much reduction in length. There is, then, only a decided meagerness of both horns. Besides these changes, other conditions may be pointed out.

In the anterolateral portion of the ventral horn, the ganglion cells are only half the normal in number and the large cells present in the upper and lower enlargements are decidedly reduced in size (table 3), and usually have a slender shape, but are seldom shrunken. In both enlargements, a small area of ganglion cells at the medio-anterior portion of the ventral horn protrudes into

the funiculus proprius anterior in the normal, but in the affected birds, it does not protrude or is quite indefinite and contains fewer and smaller cells. Fibers which run in the anterior horn are few, especially those which run toward the central gray matter.

The nerve cells in the base of the posterior horn with their fiber network are changed strikingly, about which further description will be given later under the title of Clarke's column.

The dorsal horn shows a marked reduction in its breadth in all the pigeons, especially at its base, notwithstanding the fact that it is almost as long as the normal.

In the normal specimen, at the enlargements, especially in the lower, there are usually a few large polygonal ganglion cells $22.8\ \mu$ on the average at the lateral border of the central gray matter, and sometimes they are even in the reticular formation of the lateral funiculus near the border. These cells are rarely found in the affected birds or, if present, are small and few in number. The anterior commissure which connects both halves of the cord at the level of the lower enlargement is scant, and consequently the fibers running into the anterior horn and central gray matter seem to be very few in number.

The column of Clarke. In the spinal cord of the pigeon, especially at the level of the upper and lower enlargements, we find a large group of ganglion cells with an interlacement of fiber reticulum at the base of the dorsal horn, symmetrically located and adjoining the funiculus posterior. This structure corresponds to the so-called Clarke's column in the thoracic cord of mammals. It may be well to describe a little more thoroughly this column of cells in the normal pigeon's cord, for only a few observations have been hitherto made on this structure in pigeons and other birds.

In the cervical region above the upper enlargement, there can hardly be found a well-developed structure analogous to this column; the narrow H-shaped gray matter exhibits no marked thickening at the base of the posterior horns. Only a few small round cells 5.7 to $7.1\ \mu$ in diameter not well defined from the cells in the central gray substance are visible, and the network

of fibers around the cells is not pronounced, though a few tiny bundles enter from the dorsal roots and from the funiculus posterior. Schacherl ('02) says the small size of this column in the pigeon makes it difficult to study the internal structure of the individual cells, and he says that the cell group in the upper cervical cord which corresponds to Clarke's column cannot be distinguished.

In the upper enlargement, cervical intumescencia, is found an analogous structure to Clarke's column in full development, but this is different from the arrangement in man and other mammals, for in these it does not appear at this level. It has a large spherical form on each side at the base of the dorsal horn, having its larger diameter from the medioventral to the laterodorsal side and its shorter diameter from the mediodorsal to lateroventral side. Its huge structure, 0.334 to 0.384 mm. in diameter in the average normal pigeon, occupies almost the whole space of the gray matter at the base of the dorsal horn. The marked protrusion into the white matter of the posterior funiculus as seen in man does not occur in the pigeon; the whole group of cells is within the horn or in the central gray matter and causes no bulging.

The cells are generally round, oval, sometimes polygonal; they vary in number from ten to eighteen, and among them there are three to six large cells in each average section. The size of these large cells in the normal varies from 31.3 to 51.3 μ in diameter. The area of this cell group is filled and surrounded by a mass of fine network of fibers which is mainly composed of fibers from the posterior roots and fiber bundles from the funiculus dorsalis. This network of fibers gives the dorsal and medial borders of the column a clear definition from the surrounding tissues. The ventral and lateral borders, however, are not quite so well defined, owing to the diffuse transition into the lateral proprial fasciculus or into the fiber network of the lateral portion of the central gray matter.

In the thoracic region the column decreases in size, the contained cells are less numerous and the network not so luxuriant, but in the lower enlargement, intumescencia lumbosacralis, it

appears again in good development and almost equal to that in the upper enlargement. Here in the lower enlargement the structure is less distinct than in the upper, especially in its lateral and ventral borders; its border adjacent to the central gray matter is somewhat difficult to distinguish. In the sacral region the typical columnal feature is lost, but there often remain one or two large round or oval cells at the corresponding place.

In the affected birds, at the level of the upper cervical cord, the nerve cells in the base of the dorsal horn and in the central gray matter are small and measure on the average $6.4\ \mu$, while in the normal they measure $8.6\ \mu$ in size; they are also reduced in number. The small size of the cells makes it hardly possible to describe their internal structure, but the striking narrowness of the posterior horn at its base must be remembered. In the upper enlargement, Clarke's column, which is at its maximal thickness at this level, has a decidedly small size, fewer cells and less reticulum in all affected specimens. The cells have a spindle-like slender shape and are rarely shrunken and are not at all like the large round or oval normal cells. The fibers around and in the column are few, stain weaker, are thin and short, and often they seem as if they were cut off in pieces. They run into the dorsal horn from the funiculus dorsalis or from the dorsal roots, and in the normal they may follow into the central gray matter or reticular formation in the lateral funiculus, but in the affected birds they are usually quite thin and show a short course.

At the level of the lower enlargement, Clarke's column measures, though not exactly determined, about 0.317 to 0.384 mm. in the normal and 0.200 to 0.251 mm. in the affected birds. The cells in the affected sections are small and few in number, measuring $14.2\ \mu$ in diameter. In the affected specimens these cells are really quite rare, only one to three are found in a field at the most, while in the normal three to five are found in one field, and these measure 39.9 to $51.1\ \mu$ in diameter. The cells in the affected birds have usually a slender shape and sometimes, though rarely, seem to be slightly shrunken. Fibers which go from the end of the posterior horn or into the central gray matter in the normal take a path in a direction ventrolaterally or transversely at the

lateral edge of Clarke's column. These fibers enter apparently into the lateral funiculus, while others run into the ventral horn along the lateral edge of the central gray matter. The fibers of this reticulum are fewer and shorter than normal and are hence quite difficult to make out. They do not exhibit long strands, but appear as if they were cut in short fragments. The group of fibers which run at the lateral edge of the central gray substance at this level is not present or else quite indistinct.

All the above changes of ganglion cells, however, never progress as far as total destruction or degeneration and the cell nuclei are well preserved.

Hofmann's nucleus. This was called by Brandis ('93) merely 'faserarmes Randfeld' and by Kölliker ('02) it was fully described in the spinal cords of birds and reptiles. It is located in the pigeon at the lateroventral periphery of the lateral funiculus in the shape of a crescent, having a maximal transverse diameter 0.084 mm. and a ventrodorsal diameter 0.284 mm., and contains two to four large cells of $14.7\ \mu$ in each section of the upper cervical region in our normal birds. At the level of the enlargements it varies from 0.067 to 0.084 mm. in breadth and 0.301 to 0.334 mm. in length. This structure shows almost the same condition in the affected as in the normal.

Gowers' tract. Morphologically, with the methods we used, we could hardly distinguish a tract which corresponds to the Gowers' bundle of mammals independently in the spinal cord of either the normal or affected specimens. Brandis did not exactly make the differentiation of both tracts of Gowers and Flechsig, but always considered them as the 'Kleinhirnseitenstrangbahn' in the birds. Friedländer ('98) describes these tracts in the pigeon as quite analogous to mammals in regard to their positions. One can see, however, in his illustration that only a few scattered Marchi's small black globules exist in the anterolateral area of the funiculus lateralis, while many apparent Marchi's black globules are present in the dorsolateral portion. Kühn and Trendelenburg ('11) report that the two direct cerebellar tracts can only be well distinguished at the places near to their origins and not in their paths up the cord.

If this tract runs through the anterior commissure, as Kühn and Trendelenburg contend, our findings of a decided reduction in the anterior commissure with a decreased number of cells in the central gray substance must show the defective development of this tract, at least at its origin.

Nissl preparations. The section at various levels of the cord which were stained with toluidin blue and counterstained with erythrosin give almost the same results as indicated in reference to the general conditions of the ganglion cells. The cells in the anterior horn and in the base of the posterior horn (Clarke's column) in the upper and lower enlargements reveal a marked reduction, from one-half to two-thirds the normal, both in reference to size and number. The Nissl bodies stain a relatively light purple color in all ataxic birds, so that although the bodies can be easily distinguished they usually appear more or less diffuse and indistinct as the result of a less number and weaker staining properties when compared with the normal numerous Nissl bodies of a deep purple color. At different levels of the cord, in all affected specimens, this is observed and easily recognized in both the enlargements, where the cells are large enough for minute examination of intracellular conditions. Complete destruction, disappearance, and disintegration of Nissl bodies or the typical grouping of this substance in one part of the cell body could not be found in any of the cells. This, then, indicates that the Nissl bodies are reduced throughout the affected birds, but further than this there is nothing which resembles new or old degenerative processes.

Pia mater, blood-vessels and spinal roots. The pia mater is not thickened, but sometimes it is slightly thinner in the affected birds; the blood-vessels in the periphery of both the anterior and posterior medial sulci have a smaller caliber than normal (the largest diameter is 0.050 mm. in the affected and 0.0835 mm. in the normal on the average), but their coats show no thickening or infiltration. The capillaries and small blood-vessels appear in cross-section of the cord definitely in less number in the sections of the affected pigeons than in the normal. This may indicate an insufficient supply of blood to the spinal cord of the affected pigeons.

No round-cell infiltration is seen surrounding the central canal. Sometimes there seems to be a slight increase of neuroglia at the periphery along the medial sulcus in the funiculus posterior in a few sections; however, this is not seen in successive series of preparations. Lissauer's zone and both spinal roots in all the affected birds show no evident difference from the normal.

2. Cerebellum

The cerebellum of the pigeon lies as a dorsal cover of the fourth ventricle and anteriorly it is attached closely to the large optic lobes. It has a spherical shape and is supported on both sides by a stalk, the crus cerebelli ad medullam (Stieda, '69). This is the only cerebellar peduncle the pigeon has, any independent structure corresponding to the other two peduncles in mammals is not defined, at least macroscopically. The lamellae of the cerebellum, which run transversely on the surface, converge in the crus cerebelli, fan-shaped at the lateral part of the cerebellum. The lateral hemisphere is not present in the pigeon. As to the division of the vermis, Shimazono ('12) divided it into two parts, the vermis anterior and posterior; Ingvar ('18) lately divided it into the lobus anterior, medius, and posterior. The anterior, Ingvar divides from the medius by fissure primarius and the posterior from the medius by the fissure prepyramidalis. He came to this division as a result of his phylogenetic and ontogenic studies. The lobus anterior is divided into four lobuli; lingula, lobus centralis, and culmen, and the lobus posterior into three lobuli; uvula, nodulus, and pyramis. The interplaced lobuli between the anterior and posterior comprise the lobus medius which consists of three lobuli.

A small appendix from the posterolateral portion of the cerebellum turns up, making a furrow between it and the cerebellar body; it is shaped like an auricle, and is called the lobus lateralis or auricle. Many authors agree that it corresponds to the floccular body in mammals. The auricle consists of two main lamellae, the one large anterior, the paraflocculus, the other

small posterior, the flocculus. The former is continued from the uvula and one part of pyramis, while the latter comes from the nodulus.

A majority of the specimens were cut transversely and therefore at first there appears in the sections the lobuli of the lobus posterior, then the middle part of the vermis and the medullated cerebellar body, and at last the lobus anterior comes into view. Here it must be noticed that in the affected cerebellum, owing to its small size, when we start to cut sections from the same surface at the same time with a normal cerebellum, both being at the same level in a piece of paraffin mounting, we reach the white substance of the affected cerebellum with the knife before we reach to the same white substance of the normal.

Each lobule of the cerebellum of all the affected birds was measured and compared with that of the normal birds. The chief result is the greater or less reduction of the cortex cerebelli, especially of the molecular layer in almost all parts of the cerebellar surface. This is found to be true in all the affected pigeons. The reduction of this molecular layer is general; the vermis anterior, medius, posterior as well as auricle are generally affected. We can hardly deduce any rule concerning the situation of the reduction of this layer except that in all cases it is somewhat marked and constant in the lateral gray matter of the central medullary substance (Kleinhirnkörper) which will be described later. In none of the affected birds is there ever found any place where the molecular layer is totally absent.

The granular layer and the medullated layer are usually slightly narrower than normal, but the difference is not so striking as in the molecular layer. The granule cells present normal character. The Purkinje cells show generally no change either in size or in number. No cell is seen that has undergone any process of disintegration. The arrangement of the Nissl substance in the cell is almost normal, though it appears in much less quantity when compared with that of the normal.

The nucleus cerebellaris lateralis appears in the section of the cerebellar body at the level where the intrabulbar vestibular

fibers appear in the medulla oblongata. This nucleus is composed of many large and small groups of cells and is supposed to correspond to the nucleus dentatus of mammals (Wallenberg, '98). We may divide this into five groups, according to Shimazono, though each division is not sharp, and sometimes it is difficult to define each part exactly, especially the cell group located ventrally near the base of crus cerebelli, where the arrangement of cells becomes diffuse and they pass over into the cell group in the dorsal area of the acoustic field. No remarkable differences of these nuclei in the affected birds can be detected from the normal.

The nucleus cerebellaris medialis which is located proximal to the former nucleus shows a large round form at its maximal development. This is said to correspond to the nucleus fastigii of mammals and is said to give origin to the efferent cerebello-spinal tract (Shimazono, '12). It measures 1.035 mm. in transverse and 1.202 mm. in the dorsoventral diameter; the size of the ganglion cells varies from 26.5 to 35.3 μ in the average normal pigeon. The measurements of the same in the affected birds show no changes. It is of the same size and contains just as many cells.

At the level of these cerebellar nuclei, the medullated cerebellar body appears distinctly and it has a direct connection with the medulla oblongata, through the cerebellar peduncle. The lateral portion of the cerebellar body, notwithstanding the normal size of the cerebellar nuclei as already stated, is reduced in area both in reference to fibers and gray substance. The myelinated fiber mass just lateral to the nuclei measures 0.418 to 0.585 mm. on the average in the affected birds, while in the normal it measures 0.668 to 0.752 mm. Individual fibers stain a more or less light blue color in the affected birds, while in the normal they stain a deep purple-black; the myelin sheaths themselves are also thinner. This is true in all the affected specimens. Nevertheless, no positive proof of degeneration of the processes can be made out.

The molecular layer at this ventrolateral part of the vermis is reduced in breadth in all affected birds sometimes one-half

to two-thirds or even one-third of the normal and often shows an irregular breadth, so that it forms a wave-like layer. Measurements of this layer here vary from 0.150 to 0.251 mm. in affected and 0.167 to 0.418 mm. or sometimes even to 0.501 mm. in the normal. The granule cells reveal no changes save in some cases they seem to be rather loosely arranged. The Purkinje cells here, though they have generally almost the same appearance as in the normal specimen, yet show a slight difference. They are 26.5 to 29.4 μ long and 14.7 to 17.6 mm. wide in the normal on the average, but in the affected birds they are usually a little smaller and slender in shape. In pigeon no. K207 they are 20.5 to 26.5 μ in length and 11.8 to 14.7 μ in width. The processes or dendrites of the Purkinje cells which run perpendicularly to the surface of the cortex, having a measurable length of about 58.8 to 73.5 μ in the normal specimens, while they are short and very often difficult to make out in the affected birds.

In the sections which were cut horizontally, we can see that the affected birds have not only a narrow molecular layer, but also shallow sulci, and consequently the depth of the gyri is often reduced to one-half to two-thirds of the normal. In addition to this, when sections were being cut it could be observed that in the affected specimen one reaches the base of the myelinated layer where it branches to go to the different gyri sooner than in the normal birds.

There is no abnormal increase in cells in the cortex, especially in the molecular layer. No thickening of the pia mater is recognizable; the capillaries in the cortical layer seem not to be as numerous as in the normal.

From the above findings in the cerebellum we may easily conclude that the nucleus cerebellaris medialis and lateralis do not play a very important part in the affection observed in the birds. The chief changes consist, however, in a diffuse reduction of the molecular layer on the whole surface of the cerebellum and especially in a reduction in the lateral myelinated portion of the cerebellar body with its cortical layer near the cerebellar peduncle. In reference to the cerebellar peduncle, further description will be given later.

3. Brain stem

The medulla oblongata of each of the pigeons, both affected and normal, with the attached half of the cerebellum was studied in the successive serial sections from the distal end up to the height of the oculomotor nucleus in the midbrain. Since the normal structural relations of nuclei and tracts are not very well known, I have described, of necessity, the normal structure first before attempting to point out any alteration or difference in the affected birds.

The distal portion of the medulla oblongata. At this level of the medulla oblongata, the gray substance of the posterior horn increases in its horizontal width, the axis of the horn is directed laterally, and it contains many myelinated fibers. The myelinated fibers in the posterior horn converge ventromedially at the neck of the horn, whence they run either into the funiculus lateralis, or the anterior horn, while more proximally they also enter the anterior commissure.

The anterior horn is narrower than in the cervical cord; its axis nears the midline, and it contains an abundance of fibers which go to form a part of the thick anterior commissure. The anterior commissure also receives fibers from the lateral funiculus and from the posterior horn. These fibers in the anterior commissure disappear in the opposite medial edge of the funiculus anterior. Going more proximally, the anterior horn becomes smaller and there appears the nucleus hypoglossus, while laterodorsally to the central canal the beginning of the vagus nucleus is seen.

The fibers in the anterior funiculus correspond to the fasciculus longitudinalis medialis of mammals. This has been established by the early formation of myelin sheaths and the pathway taken by these fibers to the midbrain (Brandis, '93). Many fibers in the funiculus anterior and lateralis, which cross in the raphé and run laterally and dorsally, correspond to the internal arcuate fibers of mammals. The thick fiber bundle which runs along the ventral periphery of the medulla forms the external arcuate fibers and makes one of the important connections with the cerebellum.

In the affected birds, the gray matter of the posterior horn is small, both the transverse and ventrodorsal diameters being reduced. Moreover, it contains few cells and few and thin myelinated fibers. The fibers in the posterior horn run to the reticular formation in the lateral funiculus, while those to the anterior commissure are diminished in number, and are often hardly distinguished in the affected specimens. The two small areas of gray matter in the ventral base of the posterior funiculus, the one lying medially and the other laterally, also are reduced and contain fewer and smaller cells in all ataxic sections by exact measurement. These areas which were called by Brandis merely as 'faserfreie Stellen,' in all probability correspond to the nuclei funiculi posteriores of mammals from their similar localization and form. The fiber bundles dorsal to the posterior horn and the two small areas noted above are reduced; the fibers have a small caliber, thin myelin sheaths, and stain lighter in all affected sections. No positive degenerative processes are exhibited.

The fiber area of the ventrolateral portion to the posterior enlarged horn in the funiculus lateralis has not a distinct boundary from the neighboring fiber bundles at this level even in the normal pigeon, although it seems to be reduced in area and in the caliber of the individual fibers in the affected birds. In the anterior funiculus the gray matter becomes smaller as one passes proximally. The nuclei hypoglossi do not exhibit much difference from the normal.

A little further proximally, laterodorsal to the central canal, there appears the vagus nucleus, which shows its structure at the inferior portion of the floor of the fourth ventricle where the central canal opens into the ventricle. This nucleus is well developed as well as its transversely running intramedullary fibers in both the affected and the normal specimens. The fasciculus solitarius is quite the same in both.

In the ventral area of the medulla oblongata, along the midline there appears a long triangular area of longitudinal fibers, its base directed dorsally. This is nothing but the fasciculus longitudinalis medialis and predorsalis or 'Vorderstrangrest'

(Brandis, '93 a; Winkler, '91; Wallenberg, '03). This area of fiber tracts is not so well defined in regard to the separate fibers as in the normal, owing to the poor development of the myelin sheaths and small size of the fibers. This is one of the striking differences in all affected birds from the normal. The internal arcuate fibers which appear more proximally in the medulla oblongata are less distinct than those of the normal; this is especially easy to see at the raphé, where they decussate. The thickness of the external ventral arcuate bundle, which runs ventrally along the periphery of the medulla, gathering fibers from the posterior and also lateral funiculus, is reduced by one-third of the normal; the individual fibers are much thinner in reference to the myelin sheaths and are also reduced in number. This finding makes another striking difference in all affected birds not only at this level, but also at other levels of the medulla.

The level of the nucleus olivaris inferior. The nucleus olivaris inferior in the pigeon appears at the level of the hypoglossal root and lateral to it, but it is partly pierced by this nerve in the ventral region of the medulla oblongata. It lies in a transverse position, having a thick gray mass at the lateral end. Brandis only described "ein grosses fast faserfreies Feld" at this level without paying any further attention to the olivary nucleus. Yoshimura ('10) found experimentally that by injury to the cerebellum this nucleus degenerated almost totally contralaterally. The olivary nucleus is connected by way of the arcuate fibers to the cerebellum homo- and contralaterally and has a direct connection with the spinal cord. The median part of this nucleus contains a rich fiber network with few cells. Shimazono infers from his experimental and embryological study that almost certainly fibers from the olivary nucleus cross in the raphé and go dorsally to the cerebellum.

In all our preparations except only the case of the pigeon no. K207, the nucleus olivaris inferior is poorly developed; the nucleus is small; the normal measures transversely 0.668 mm. and ventrodorsally 0.367 mm., while the affected specimens measure 0.418 mm. transversely and 0.117 mm. ventrodorsally.

The number of contained cells is reduced, forty in each section on the average in the normal, twenty-five on the average in the affected; the size of the cells is below normal, the normal cells measuring 19.9 to 22.8 μ in diameter, but the Nissl bodies show no difference from the normal. The interolivary fiber bundle is markedly reduced; in the affected pigeon no. K158, its ventrodorsal thickness measures 0.0835 mm. instead of 0.2505 mm. as in the normal. This fiber bundle, therefore, is one-third the normal thickness. In other affected birds this reduction is also seen, but not so markedly. In the normal specimen the fibers surrounding the olivary nucleus, the circumolivary fibers, especially those dorsal and lateral to it, are thick and well developed. We see also a fiber bundle which runs from the olivary body or from its surroundings, dorsally and laterally to the spinal root of the trigeminal nerve. Further proximally this bundle appears at the medial part of the tractus spinocerebellaris and enters the cerebellum with it. This fiber mass appears less definite and stains lighter than the normal deep color, so that the distinctness of the boundary of the gray matter of the olivary nucleus seems to be less defined in the affected Weigert preparation than in the normal sections. This is due to the scantiness of fibers and thin myelin sheaths around the nucleus. The fibers which are seen within the nucleus are also diminished in number.

The internal arcuate fibers are few in number and do not stain as deeply as the normal, and the scattered ganglion cells in the reticular formation are small and not nearly as numerous as in the healthy birds. The restiform body is markedly reduced, 0.200 to 0.251 mm. in the affected and 0.367 to 0.418 mm. in the normal. The fiber mass which runs longitudinally at the ventromedial region of the restiform body is not stained as well as in the normal and contains fewer fibers. No difference is recognizable in the spinal roots of the trigeminal nerve in either its staining qualities or its dimensions. Fibers of the hypoglossus and the cells in the nucleus n. hypoglossi have all a normal structure.

The zone of *fibrae arcuatae externae ventrales* is extremely thin and contains only a few fibers and stains less intensely than normal. The area of the *fasciculus long. med.* and *predorsalis* shows diminished transverse as well as ventrodorsal dimension, especially the former. In the distal portion of the medulla oblongata, this *fasciculus long. med.* cannot be separated from the fiber tract which lies ventrally to this fasciculus. The area of this fasciculus contains many smaller fibers, all more variable in size than the normal; the average caliber is $5.7\ \mu$ in the ataxic and $8.6\ \mu$ in the normal; the staining also not so deep as in the normal. Nucleus of the *ala cinerea* and the fibers of the *vagolossopharyngeus* are normal.

All above findings are quite uniform in all affected pigeons, there being only a difference in degree.

The level of the cochlear nuclei. The acoustic nerve enters the medulla oblongata in two roots as in mammals; the one is the dorsal, distal, or lateral root, the *nervus cochlearis*; the other is the ventral, proximal, or medial root, the *nervus vestibularis*. The nuclei of the acoustic nerve are divided into three main groups: One, the nucleus angularis, 'Eckkern,' located at the dorsolateral portion of the medulla, into which the cochlear root enters; this corresponds to the *tuberculum acusticum* of mammals (Brandis, '94; Winkler, '91). The second nucleus appears in the lateral wall of the fourth ventricle, crescent-shaped, surrounded by a medullated fiber mass, and is named the nucleus parvo-cellularis, 'der kleinzellige Kern.' The third one, the largest, occupies the space between the above two nuclei, nucleus magno-cellularis, 'der grosszellige Kern.' The magno-cellular nucleus is supposed to be analogous to the nucleus Deiters in mammals.

The nucleus angularis as well as the cochlear stem in all four ataxic birds are almost the same in the normal in reference to their developmental conditions. The nucleus parvo-cellularis has sometimes a little narrower shape and the medullated fibers around it appear to be slightly reduced. The nucleus magno-cellularis, though it appears almost the same as normal, seems to be diminished at its proximal dorsal portion in the

base of cerebellar peduncle, the size of each cell, however, is not changed; the large cells in this area are 39.9μ in diameter in both the normal and affected. These reductions of two nuclei, nevertheless, are by no means striking. The so-called 'Bogenzug' of Brandis or the dorsal fiber system of the n. octavus in the acoustic area runs from or around the nucleus parvocellularis partly to the raphé and partly dorsally to the cerebellum. Both fiber paths of this bundle are sometimes slightly thinner in the affected cases, though there is no striking variation from the normal. The fibers that are around or come from the nucleus magno-cellularis and that run dorsally to form the medial part of the cerebellar peduncle and that then pass across the peduncle obliquely to enter the cerebellar cortex exhibit no differences from the normal.

A fiber bundle which runs dorsomedially to ventrolaterally, from the ventrolateral border of the nucleus parvocellularis to the small round nucleus medial to the spinal root of the n. V. appears plainly at the more proximal level, where the sensory nucleus of the trigeminal nerve appears. This fiber bundle and nucleus appear quite the same in both the affected and healthy birds. They seem to correspond to the beginning portion of the ventral system of the central acoustic nerve, as was brought out by Wallenberg ('98). The further cerebralward course of fibers from this nucleus which cross the abducens root at its ventral two-thirds is not easily differentiated from internal arcuate fibers in any of our normal or ataxic preparations.

The restiform body at this level is much more apparently reduced; its size is often one-half the normal, on the average the transverse diameter is 0.200 to 0.251 mm. in the affected, and 0.334 to 0.418 mm. in the normal. The fiber mass cross-sectioned just medioventral to the restiform body is not as large as in the normal section. The cerebellar peduncle appears at this level connecting the cerebellum and medulla oblongata in frontal section. The peduncle is markedly reduced in its whole transverse breadth; 1.336 mm. in the affected and 1.754 mm. in the normal. This reduction is due to the thinness of

the spinocerebellar tract and bundles from the arcuate fibers which form the main portion of the restiform body, and is observed in all serial sections in all the affected birds.

The fiber bundle along the ventral periphery of the medulla is quite thin in all affected pigeons. This is indeed very striking; the ventrodorsal thickness of this bundle measures 0.100 mm. in the normal and 0.043 mm. in the affected bird—a dimension of less than one-half the normal. There is not only a reduction of thickness of the bundle, but each individual fiber is smaller and stains weakly owing to its thin myelin sheaths. The above condition of this bundle throughout its course is common in all affected birds and it constitutes one of the most decided changes in the medulla.

The internal arcuate fibers are less prominent and quite indistinct, owing to the reduction in fibers, their poor staining properties and the small size of the individual fibers; the scattered large ganglion cells between the fibers in the reticular formation are not only few in number, but also small in size, the average size of the large cells being 22.8 to 37.5 μ in the affected and 28.5 to 57.0 μ in the normal. The area of the reticular formation is apparently reduced.

The tall triangular area of the longitudinal fiber bundle along the raphé with its base directed dorsally to the floor of the fourth ventricle, that is, the area of the fasc. long. med. and predorsalis is smaller, especially in its transverse diameter, this measuring 0.638 mm. in the affected and 0.985 mm. in the normal. The fibers which cross the raphé from side to side and the fibers which run ventrodorsally in the raphé in this area are quite scant. The fibers, both the longitudinal and transversal, are thin, indistinct, and do not stain a deep black by Weigert. The spinal root of the trigeminal nerve shows a good development in both the healthy and unhealthy birds.

The level of the vestibular nerve. Proximal and a little ventral to the cochlear stem, the nervus vestibularis appears as a large bundle entering the medulla oblongata. A part of the fibers runs dorsally to the acoustic area, to the nucleus magno-cellularis, while the other and greater part of the fibers runs medially

to the raphé, where it enters into the fasc. long. med. Some of the fibers of the first group disappear into the fiber group surrounding the nucleus parvo-cellularis, giving apparently fibers to this nucleus, while others run dorsally and laterally and enter into the cerebellar peduncle. The second group of the vestibular fibers runs transversely, as already seen, toward the raphé, beneath the nucleus parvo-cellularis and the so-called 'Bogenzug' of Brandis, to enter the raphé.

The breadth of the stem of the n. vestibularis just outside of the medulla oblongata in all affected birds has the normal dimension, both being 0.585 mm. The nucleus of this stem which is analogous to the ganglion of Scarpa in mammals shows no difference from the normal. At the lateral portion of the medulla, after passing through the restiform body, the intrabulbar vestibular fiber bundle increases in its dimension; it measures on the average 0.835 mm. in the affected as well as in the normal birds.

When we pursue the vestibular fibers transversely from the periphery toward the raphé, however, we find differences in the floor of the fourth ventricle at the place where the vestibular fibers meet the fasciculus long. med. In the ataxic, the bundle of fibers which goes from a connection with the vestibular nerve fibers and the 'Bogenzug' just at the lateral side of the fasc. long. med., where they leave this fasciculus, is markedly reduced in dimension, being about two-thirds that of the normal. Both of the fasciculi long. med. in their transverse diameter measure 0.835 mm., while in the normal they measure 1.086 mm. on the average. In pigeon no. K137 the transverse diameter is only 0.718 mm. The ventrodorsal diameter of the fasc. long. med. including fasc. predorsalis, on the contrary, is not very much reduced, 1.002 mm. in the normal and 0.919 mm. in the ataxic; often both measure the same. In the area of the fasc. long. med., the fiber bundle along the raphé, running ventrodorsally, is reduced in its transverse diameter; in the affected pigeon it measures 0.08 mm., while in the normal it measures 0.167 mm. The fibers crossing transversely in the triangular area of this fasciculus are also apparently diminished in number. The longitudinal fibers are diminished and the average caliber of each fiber is 5.7μ in the affected and

8.6 μ in the normal; the staining properties of the fibers here are poor.

The fiber groups which go dorsally and to the ventral portion of the acoustic area and which pass into the ventral part of the nucleus parvo-cellularis are slightly smaller than normal. In the ventral region of the cerebellar peduncle, between the medial edge of the spinocerebellar tract and the lateral region of the 'Bogenzug,' we find an area of large ganglion cells which is the proximal process of the nucleus magno-cellularis. The transverse breadth of this nucleus here measures 0.835 mm. in the affected and 1.084 mm. in the normal; its dorsoventral boundary is somewhat difficult to make out because of the close relation to the ventral cells of the nucleus cerebellaris lateralis. The cerebellar peduncle has a small breadth and the lateral fiber mass in this peduncle which is composed of fibers that come mainly from the medulla oblongata has a breadth of 0.334 mm. in the affected and 0.835 mm. in the normal, or a ratio, therefore, of 1:2.5.

The difference of the external ventral arcuate fibers between the normal and affected becomes more prominent than ever at this point, 0.100 mm. in normal and 0.066 mm. in the affected birds. The staining properties, caliber, and other conditions of each individual fiber are the same as described before. It is clearly seen that the *fibrae arcuatae externae ventrales* run latero-dorsally and enter the restiform body and with the *tractus spinocerebellaris* pass dorsally up to the lateral edge of the cerebellar peduncle. The fibers in the reticular formation have the same changes as noted at the former level. The changes in the fasc. long. med., the external ventral arcuate fibers, the lateral portion in the cerebellar process, and the restiform body are the main important differences in the affected pigeons at this level with, however, no apparent degenerative processes in either fibers or nuclei.

The facial nerve and its nucleus appear normal.

The level of the sensory trigeminal nucleus. The spinal root of the trigeminal nerve is pierced ventrodorsally by a few fibers which come from the reticular formation and pass toward the

cerebellar peduncle dorsally. These fibers can be easily seen in the normal section, while in the affected section they cannot be seen distinctly at all. The area and staining properties of the spinal root of the nervus trigeminus are normal. In the ventral region of the cerebellar peduncle, more proximally than the acoustic area and the vestibular fibers, there appears a large oval nucleus, with the long axis from the mediodorsal to the ventrolateral portion of the brain stem. This is the sensory nucleus of the trigeminal nerve (nucleus magnus nervi trigemini, Wallenberg). In the lateral area of this nucleus there runs a thick bundle from the brain stem laterally to the cerebellar peduncle, but there are also fiber groups which run to the medial side of the cerebellum. The fiber bundles which surround this nucleus at its medial side run chiefly to the medial region of the cerebellar body, some of them, however, cross in the midline at the base of the cerebellar body. From the sensory nucleus of the trigeminal nerve fibers emerge at its ventrolateral border and pass out of the brain stem.

The nucleus with its contained cells as well as its peripheral fibers when compared with the normal reveals no abnormalities.

The three nuclei of the motor V. described by Brandis ('95) with their cells and fibers appear perfectly normal.

The fasc. long. med. and external ventral arcuate fiber bundle in all affected specimens are less well developed as at the former level. The eminences of the fasc. long. med. in the floor of the fourth ventricle are much flatter than normal. In pigeon no. K207, the fiber bundle at the ventral periphery of the brain stem is almost absent.

At the ventral part of the brain stem there is a symmetrical small nucleus, directly dorsal to the external ventral arcuate bundle. This nucleus lies medial to the intracerebral roots of the nervus abducens; it is round in shape and contains about eighteen small round cells. Surrounding this nucleus there are some fibers which cross the raphé transversely to the other nucleus. In the ataxic specimens except pigeon no. K172, this nucleus is rather flat than round, contains smaller and fewer cells and the interspersed fibers are few; the fibers around it are ap-

parently diminished in number. This nucleus appears in frontal section, and proximally disappears at the level where the trochlear nucleus shows its largest size. In the affected preparation, on the other hand, the nucleus disappears at the level just proximal to the place of appearance of the sensory nucleus of the trigeminal nerve.

This nucleus has never been exactly described, even though it seems to be similar to the oliva in Friedländer's illustration ('98) or the medial α -nucleus ('Trapezkern,' Westphal) of Wallenberg ('04) in reference to its localization. The inferior olivary nucleus of the pigeon lies, however, far distal and lateral to the hypoglossal root, while the superior olivary nucleus lies more dorsalward, as already described. If this nucleus had a connection with the fiber bundle of the ventral system of the eighth nerve, like the superior olivary nucleus and the nucleus isthmi, it would be normal as these are. Thomas ('11) reports the atrophy of the arciform nucleus in cerebellar atrophy, and contends from his experiments that the arciform nucleus has close relation with the pontine nucleus. It seems to me rather, therefore, that this nucleus may have some close physiological relation to the already described ventral fiber bundle or to the arcuate fibers than to the ventral system of the eighth nerve.

The internal arcuate fibers in the reticular formation are fewer and paler; differences especially marked in the fibers which cross at the raphé. At the lateral edge of the brain stem, a fiber mass runs toward the cerebellum passing through the cerebellar peduncle, which measures 0.25 mm. in the normal and 0.08 mm. in the affected birds. Going more proximally from this level, the fasc. long. med. becomes more and more round in section and the cerebellar peduncle decreases in breadth.

At this point it will be well to describe more thoroughly the cerebellar peduncle, from its distal to proximal end, for reduction of this peduncle is one of important differences in the affected birds.

The distal connection of the medulla oblongata to the cerebellum in frontal sections begins at the level of appearance of the cochlear nerve. The spinocerebellar tract at the lateral

edge of the medulla oblongata which hitherto ran longitudinally, changes its direction dorsalward. As one goes cerebralward, the peduncle increases in its thickness, gathering fibers from the medulla oblongata. When the wide bundle of the nervus vestibularis appears, one sees a large fiber mass running dorsally from the lateral edge of the medulla oblongata to the lateral portion of the cerebellar peduncle whose fibers go chiefly to the cortex on the same side. This fiber mass gradually increases in breadth, receiving fibers from a bundle which runs on the ventral periphery of the medulla oblongata from one side of the cerebellar peduncle to the other. This ventral fiber bundle shows its maximal thickness at the level where the cerebellar peduncle has its greatest thickness, i.e., at the proximal portion of the vestibular nerve. Regarding this fiber bundle, from its relation to the cerebellum, from its anatomical localization, and especially from its striking diminution in size (one-third to one-half the normal breadth in all the affected birds), it will not be a great mistake to indorse Stieda, who suggests that this fiber bundle in large part, if not wholly, is comparable with the pons varolii of mammals.

At the region where the facial nerve fibers appear in the medulla and the nucleus cerebellaris medialis forms a large round nucleus in the cerebellum, the fibers in the peduncle become more and more numerous, and go not only to the lateral portion, but also to the median portion of the cerebellar body, some of them crossing the midline to the opposite side. The fibers of the brachium conjunctivum appear at the medial edge of the peduncle. Then at the ventrolateral base of the peduncle, taking the place of the position of the large cell area of the acoustic field, the oval sensory nucleus of the trigeminal nerve appears, well surrounded by medullated fibers, as indicated before. We see here nothing but a rich mass of fibers in the peduncle which run to the lateral and medial parts of the cerebellar body. Farther proximally, the fibers which go from the dorsal portion of the brain stem into the velum medullare appear. At the location where the massive bundle of the sensory V. root appears, the connection between the cerebellum and brain stem is lost. After

separation of the cerebellum and brain stem in frontal sections, in the dorsolateral part of the brain stem, a thick fiber group runs up to the median line. Laterally, there is seen, outside of the brain stem, the peripheral trochlear nerve which is cut parallel to the fibers. Proximally to the trochlear nucleus, we see a massive fiber bundle which forms the ventrolateral part of the brain stem to the optic lobe.

The breadth of the cerebellar peduncle in the affected birds at different heights shows a marked reduction in its distal and middle region, with only a slight reduction, however, in its proximal region. If we measure the cerebellar peduncle at its narrowest place, namely, from the lateral edge of the cerebellar ventricle to the place where the external lateral surface bends and turns medially, we obtain the following measurements: At the distal region of the cerebellar peduncle, its breadth measures 1.837 to 2.004 mm. in the normal and 1.253 to 1.336 mm. in the affected specimen; in the middle portion of the peduncle, 1.921 to 1.754 mm. in the normal and 1.320 to 1.253 mm. in the affected, and in the proximal portion, 1.169 mm. in the normal and 1.002 mm. in the affected pigeon. Moreover, this reduction is chiefly seen in the lateral fiber bundle of the cerebellar peduncle, especially in the distal and middle portions of the peduncle.

In the cerebellar peduncle there are many other fiber bundles besides those already described. These were studied by Frenkel, Wallenberg, and Shimazono and will be briefly discussed.

The tractus octavo-cerebellaris goes up directly from the acoustic area, passing through the cerebellar peduncle to the lateral region of the cerebellar body and enters the cortex of the cerebellum. The tractus octavo-floccularis runs from the large cell nucleus and nucleus parvocellularis to the medial part of the peduncle, forming an arc obliquely to the peduncle, and enters the lobus lateralis.

The fibers in the tractus octavo-floccularis in the affected section are separated into a few small fiber groups, while in the normal they run as one solid bundle in the ventral part of the peduncle; as a result of this, the fibers in the affected specimen are reduced in volume without showing any kind of distinct degener-

ative processes in their myelin sheaths. The nucleus parvocellularis, as already indicated, does not seem to have quite the same semilunar shape as the one in the normal section and the cells appear to be a little smaller, even though the difference is not striking.

The tractus octavo-cerebellaris is seen best a little proximal to the above section of the tractus octavo-floccularis. Here the tract runs from the acoustic area dorsally, crossing the tractus octavo-floccularis, and then passes through the ventral group of the lateral cerebellar nuclei in separated small bundles to reach the dorsal part of the cerebellar body. This bundle diverges into ten to twelve fiber groups in both the affected and normal sections; the whole breadth of the bundle in the affected pigeon measures 0.114 mm., while in the normal it is 0.151 mm. No degenerative processes, however, could be seen.

The tractus quinto-cerebellaris emerges from the sensory trigeminal nucleus, passes the cerebellar peduncle medial to the spinocerebellar tract to the cerebellar body. This tract is well seen in both birds and there is no recognizable difference between them. The sensory nucleus of the trigeminal nerve in the affected birds is in good condition, as already observed.

The tractus bulbo-cerebellaris runs from the nucleus lateralis, located ventral to the facial nerve stem in the peripheral border of the medulla, to the lateral side of Deiter's nucleus dorsalward to disappear in the tractus spino-cerebellaris in the cerebellum. This tract is not easily differentiated in either our normal or affected sections, but it must be included in the reduced lateral fiber groups of the medulla or the cerebellar body. The tractus cerebello-spinalis, an efferent tract, which should be in the cerebellar peduncle, is difficult to define, but so far as the cerebellar nucleus (medialis) is concerned, which is supposed to be the nucleus of origin, there is no change in it whatever.

The tractus cerebello-mesencephalicus enters the brain stem at the ventral region of the cerebellar peduncle and runs medianward. Wallenberg and Shimazono proved this by injury of the nucleus cerebellaris lateralis. In our sections this tract, which is the brachium conjunctivum, appears medial to the sensory

nucleus of the trigeminal nerve as a fiber mass running from the ventral part of the cerebellar peduncle to the medial part of the medulla. This brachium is perfectly normal in the affected specimens. The decussation of the brachium as well as the nucleus ruber at the proximal region of the oculomotor nerve are well developed.

As to the connection of the cerebellum to the fasciculus longitudinalis medialis, no definite structure could be identified.

The levels of the trochlear and oculomotor nuclei. The trochlear nucleus, its size, shape, and number of cells, together with the fibers coming from the nucleus in the affected specimens, all appear as in the normal sections. The whole dorsoventral diameter of the brain stem at this level of the nucleus trochlearis measures in the midline 3.507 mm. in the normal and 2.922 mm. in the affected section. The entire width of the brain stem from side to side measures 5.511 mm. in the normal and 4.843 mm. in the affected. These measurements show a reduction of the brain stem at this level, but the small size is chiefly due to a diminution in area of the reticular formation. The ventral arcuate bundle has already disappeared.

The oculomotor nucleus appears proximal to the trochlear nucleus as its continuation, medial and slightly dorsal to the fasc. long. med. The dorsolateral centers of this nucleus contain small well-defined cells, but the other centers have large cells. From the nucleus fibers emerge passing ventrally, part of them crossing the raphé, where they form the internal fibers of the opposite oculomotor nerve. At the point of emergence of the nerve fibers from the brain stem, there appears in the nerve stem a half-moon-shaped, colorless line in the Weigert preparation. This condition may be a structure analogous to that already discussed in the cranial nerve roots in the human by Thomsen ('87), Hülles ('06) and Staderini ('90). No pathological or defective development in the oculomotor nerve fibers or in its nucleus is visible in the affected pigeons.

The fasc. long. med. in this region is small and appears less oval than the normal, but the reduction is not as marked as at the former levels; it measures transversely 0.317 mm. in the affected,

0.367 mm. in the normal and 0.334 mm. in the affected, 0.468 mm. in the normal dorsoventrally. The nucleus ruber with the decussation of the brachium conjunctivum, are both quite normal in size and in number of cells. The cells in the red nucleus measure on the average 28.5μ in both the normal and affected sections.

In the nucleus isthmi in the brain stem and nucleus mesencephalicus lateralis in the optic lobe, no distinct difference from the normal can be detected.

SUMMARY AND DISCUSSION

We may summarize here the chief differences observed in the affected birds. Macroscopically, the central nervous system especially, cerebellum, medulla oblongata, and spinal cord are reduced in size in all the affected birds. A reduction in weight, chiefly in the distal portion of the brain, is present both absolutely and relatively in reference to the body weight and the weight of the whole brain in comparison with those of the normal.

In the cerebellum, the molecular layer is decidedly reduced in its thickness, showing microgyri throughout. Though it is established that the direct spinocerebellar tract distributes its terminals in the ventral lobuli of the anterior lobe (Ingvar, '18), no localization in regard to the reduction in the cortex of the cerebellum in our cases could be seen. The reduction of the cortex in the lateral part of the cerebellar body, however, is relatively striking and also is constant in all affected birds. The Purkinje cells, even though they exhibit sometimes a little more slender shape and shorter dendrites, show no decided regressive alterations. Neither is there seen a total defect or any increase in the nerve elements in the cortical layer. The medullated layer is paler by the Weigert, owing to the thin myelin sheaths of each fiber, but not from swelling or segmentation. The large nuclei in the cerebellum, the nucleus cerebellaris medialis and lateralis, as well as the tractus cerebello-mesencephalicus, are in as good condition as in the normal. The pedunculi cerebelli show striking reduction, sometimes to one-half the normal. Thus is well seen in the inferior and middle portion of the peduncle, but not so

marked in the anterior portion. The fibers from the reticular formation to the peduncle are hardly recognizable.

In the medulla oblongata, the most conspicuous and constant changes in all affected birds are the reduction of the fiber bundles in the ventral periphery of the brain stem, the fasciculus longitudinalis medialis, the cerebellar peduncle, and the reticular formation, including the internal arcuate fibers and ganglion cells. Other differences, though not so considerable as in the above structures, and sometimes not constant, may be pointed out: the reduction of the nuclei funiculi posteriores, nucleus olivaris inferior with its inter- and circumolivary fibers, and a symmetrical nucleus which is located at the ventral periphery in the middle portion of the brain stem. All the cranial nerves are normal in nuclei and fibers in all affected birds. The nucleus ruber, the brachium conjunctivum, the nucleus isthmi (nucleus lemniscus lateralis) in the midbrain, and the nucleus mesencephalicus lateralis in the optic lobe appear in good condition.

In addition to the small size of the gray and white matter in the spinal cord en masse, there is decidedly poor development of cells in Clarke's column, the anterior horn, and the central gray matter, all those present being small in size, often one-half the normal, few in number, with scanty processes. The median portion of the funiculus anterior, the direct spinocerebellar tract, and the posterior funiculi are reduced in area and also show indistinct borders owing to the intermingling of abnormally small and delicate fibers which show a diminution of caliber. The fiber network in all the gray matter, especially, in Clarke's column is scant. The capillaries and small blood-vessels in the substance of the spinal cord and cerebellar cortex are apparently greatly reduced. None of the spinal roots or Lissauer's zone show any variations. In all cases there is never seen a definite degenerative or regressive process, such as segmentation of myelin sheaths, or increases of the neuroglia or interstitial tissues. No thickening of the pia mater, the coats of the blood-vessels, or abnormal cell infiltration is observed.

Thus, the changes in the central nervous system in the affected pigeons may be regarded as a hypoplasia or developmental inhi-

bition in the proprioceptive system, part of the motor system, and some structures connecting the medulla and cerebellum, occurring during growth with scarcely any definite degeneration.

It is not an easy problem to explain from our specimens what part of the cerebellum or spinal cord may have been primarily interfered with in its growth. Again, it is more difficult to explain why just those neurons are especially affected. Williamson ('11) holds the opinion that a tendency for alteration in the peripheral parts of the cord may result from the fact that this area has a poorer blood supply than the central. Pitt says there is an inherited tendency toward general early vascular deterioration in certain cases, while Gowers suggests that abiotrophy, or an inherited tendency toward early death of the nerve fibers may occur in this condition (Turner, '10). Though these explanations have been given to account for the changes observed in hereditary ataxy, none quite fit in with our cases. The apparent scantiness of the blood-vessels and the capillaries in the cerebellar cortex and in the spinal cord in our cases might possibly have some relation to this question, yet this condition of the vessels is far from sufficient to explain all. We are, therefore, as yet entirely ignorant as to why just these systems should be picked out.

The question then arises as to how these symptoms could develop without any degenerative or regressive processes. Since certain groups of neurons in the central nervous system are improperly developed while others are almost normally developed, there results an unbalanced arrangement in their functions in the early life of the bird which may appear as a disturbance of coördination. In this, our cases seem to be similar to some extent, but not quite the same as the case of Nonne ('05), who reported a congenital small central nervous system in the human without degeneration, associated with cerebellar symptoms which Holmes ('07, '07 a) classified as his sixth type of hereditary ataxia from the standpoint of pathologic anatomy. The fact that the original ataxic pigeon no. 151, according to the record (Riddle), recovered from the disturbance when an adult, seems to me to give a sort of confirmation to the above view.

Under the name of hereditary ataxia, we understand a condition of congenital disturbance of coördination of movements or even in standing, static ataxia. This disease is generally classified into two types: one is the Friedreich's ataxia and the other is the L'hérédo-ataxia cérébelleuse. The former was first described by Friedreich in 1863, who noticed a typical hereditary form of a chronic degenerative atrophy in the posterior and lateral funiculi of the spinal cord. The affection is characterized clinically by a disturbance in the coördination of movements, as he says: "Die Krankheiten dürfte vom klinischen Gesichtspunkte aus als chronische progressive Lähmung der Combination der Bewegungen, von pathologisch anatomischen Standpunkte aus als chronische degenerative Atrophie der spinalen Hinterstränge zu bezeichnen sein." After his discovery of this affection, similar cases were reported by Carpenter ('71) and Gowers ('80) in England, and Brousse in France ('82), who had proposed to call the affection by the name of Friedreich's disease (Marie, '92). The chief changes in this disease are usually a thin small cord, with degeneration or atrophy, and, consequently secondary sclerosis of the lateral and posterior columns, and thickening of the pia mater. The frequently affected tracts are in the proprioceptive systems in the cord, namely, the direct spinocerebellar tracts and the columns of Goll and Burdach, but sometimes the lateral pyramidal tract is also involved. Occasionally there is more or less diminution in the number of fibers in the anterolateral column, and also atrophy of the cells in both the anterior and posterior horns (Blocq and Marinesco, '90; Barker, '03). The cells of the column of Clarke are notably degenerated or else very poor in development. All authors agree in that this disease is due to an arrest of development or growth of the various systems of fibers in the spinal cord (Menzel, '91).

Under the title of L'hérédo-ataxia cérébelleuse, Marie ('93) reported two cases of a familial ataxia, and collected from the literature a series of similar cases, in which atrophy of the cerebellum was found. He has established a symptom-complex distinct from Friedreich's ataxia. Marie's disease is believed to be

due to the congenital atrophy of the cerebellum, mostly a maldevelopment of the cortical layer, often with atrophy of the molecular, granular layers and also Purkinje cells. The reduction or degeneration of the inferior and middle cerebellar peduncles is usually cited (Menzel, '91).

Many transitional types of these two diseases, however, have been reported afterward in reference not only to symptomatology, but also to pathological anatomy (Mingazzini, '05). Many authors (Oppenheim, '00; Holmes, '07; Jendrassik, '11, and others) at present believe these two diseases to be merely one type of hereditary ataxia in which there may at times be a prominence of spinal-cord involvement while at another time the cerebellar involvement predominates, according to the main localization of the affection.

It is evident from the indicated pathological findings found in the central nervous system, as well as from symptoms exhibited during life and from the detailed family history, that the disease of our pigeons is familial and congenital and that it comes quite in accordance with the so-called hereditary ataxia of man.

The condition of static ataxia and the modes of gait from '*démarche ébrieuse*' to somersault may be considered as a cerebellar disturbance or, if not, they must have at least a close relation to some important tracts, such as the direct cerebellar tracts, which anatomically have a connection with the cerebellum. On the other hand, the deformity in the toes found in three affected birds seems rather to point to the so-called Friedreich's foot or *pes cavus* which has never been recorded in a case of simple atrophy of the cerebellum (Marie's disease), but is no doubt a symptom arising from a lesion in the spinal cord.

Thus our subjects are a type in which the spinal cord and the cerebellum as well as the medulla oblongata are involved, presenting on one hand Friedreich's ataxia and on the other hand Marie's disease at the same time from both the symptomatical and pathological points of view. Had we examined the spinal cord only, it might have been a typical case of Friedreich's ataxia, whereas the study of the cerebellum alone would have given us that of Marie's disease. This combination of the symptoms and

pathological changes accords with the authors who believe in the similarity of these two kinds of hereditary ataxia in human cases.

As to the real etiology of this disease, it has been assumed that many factors may play a part, such as idiocy, epilepsy, alcoholism, acute infectious diseases, and consanguinity of the parents (Oppenheim, '00; Starr, '09), but none of these have much real value. The connection cannot be made. They lack positive proof as etiologic factors.

As circumstantial pedigrees show, all our cases descend from an original egg (pigeon female, no. 151) produced by the weakening influence on a reproductively overworked normal parent (from records of Dr. Oscar Riddle). Whether or how this overwork has a direct influence on the central nervous system or exerts some secondary effect on this system as a result of some nutritional changes is a further difficult problem. Nevertheless, we feel that we can deduce the very interesting fact that some disorder or disturbance caused by 'reproductive overwork' was at least an important etiological factor of this disease, and that the basis for such a conclusion in the present case does not rest on uncertain observations, as has often been done previously, but upon a practical and experimental foundation.

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El desarrollo de los ganglios craneales del simpático en la rata.

El presente trabajo trata especialmente de los ganglios del simpático que se relacionan durante el desarrollo con los nervios craneales situados mas caudalmente (nervios facial, glossofaríngeo, y vago), tratando el autor de llenar el aparente vacío que existe en la cadena morfológica de los ganglios craneales de esta región. Las interpretaciones antiguas se discuten con algún detalle. Los resultados de este estudio indican el origen total o, al menos parcial, de los nervios del simpático: cardíaco, esofágico, traqueo-esofágico, pulmonar, gástrico e intestinal, a expensas del vago. Las células nerviosas de origen glossofaríngeo originan los ganglios del tercio posterior de la lengua, ganglios faríngeos, ciertos ganglios del plexo timpánico y el ganglio ótico. El ganglio eseno-palatino es un ganglio del ramo palatino VII (nervio petroso superficial mayor). Se nota por esta causa una estrecha relación entre el modo de origen de los ganglios ótico y eseno-palatino y el curso de sus fibras preganglionares. Las pruebas acumuladas por el autor están en favor de la interpretación de los ganglios submaxilares y sublinguales, juntos con los pequeños ganglios de los dos tercios anteriores de la lengua, como originados a expensas del facial por medio de la cuerda del tímpano. El ganglio ciliar se deriva exclusivamente de elementos del trigémino. Las células ganglionares del nervio terminal se originan por proliferación de las células de la fosa olfatoria. Las células ganglionares del simpático presentes en el plexo carotídeo interno y plexos aliados, junto con ciertos ganglios del plexo timpánico, son crecimientos anteriores del ganglio cervical superior del simpático.

Translation by José F. Nonidez
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THE DEVELOPMENT OF THE CRANIAL SYMPATHETIC GANGLIA IN THE RAT

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THIRTY-SIX FIGURES

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INTRODUCTION

The developmental history of sympathetic ganglia, associated with the various cranial nerves, has hitherto been little studied. The attention of embryologists has been almost entirely directed toward determining the origin of the larger sympathetic ganglionic masses, namely, the ciliary, sphenopalatine, otic, and submaxillary ganglia. The general consensus of opinion would seem to assign to the last three ganglia a trigeminus origin, at least in mammals. Investigation of the origin of the ciliary ganglion has been productive of varying results. Practically nothing is known concerning the development of sympathetic ganglia related to the more caudally situated cranial nerves, the facialis, glossopharyngeus, and vagus. The tendency is to consider definitive sympathetic ganglionic masses absent. The neural crest, however, is continuous throughout the region of the cranial

nerves, at least as far anteriorly as the trigeminus. Since sympathetic ganglia are known to be developed in relation to each segmental spinal nerve and likewise possibly in connection with the trigeminus of higher forms, their apparent absence in the case of facialis, glossopharyngeus, and vagus leaves a curious break in the morphologic chain of ganglia. With these facts in mind, the writer undertook, some three years ago, a reinvestigation of the origin of sympathetic ganglia in connection with the cranial nerves, the facts ascertained being embodied in this paper.

HISTORICAL

Numerous efforts have been made to account either for the presence or absence of sympathetic ganglion cells developmentally related to the facialis, glossopharyngeus, and vagus nerves. Previous works dealing with the subject may be classified under two general headings: first, those admitting and explaining the absence of such cells; secondly, those seeking to recognize either in the cerebrospinal ganglion or in the central nervous system, a representative of the absent sympathetic ganglion. These two general groups of interpretations obviously overlap.

Perhaps the sole representative of the first of these groups consists in a brief paper by Ariëns Kappers ('08). If I am correct in my interpretation of Kappers, his reasoning is as follows: From numerous studies, both developmental and comparative, Kappers has concluded that there exists in the central nervous system some relation between the direction of migration of the cell body of the neuron and the source of maximum stimulation; the cell body tends, namely, to move in the direction whence maximum excitation proceeds. In the sympathetic nervous system we are dealing with a migration of cell bodies in a direction apparently exactly the reverse of the direction-type observed in the central nervous system. This variation is to be explained through the observations of Langley and Anderson ('94) and afterward Langley alone (numerous papers, '99, '00, '03) on the physiology of the axon-reflex. Kappers ventures to suppose that the axon type of stimulation is

the type most prevalent in the sympathetic nervous system, and that consequently the direction of migration of cell bodies of sympathetic neurons forms no exception to the expressed generalization.

In the application of this principle to the problem of the head sympathetic, Kappers draws certain conclusions: In fish forms the possibility of stimulation of sensory endings of cranial visceral nerves is in excess of the possibility of stimulation of spinal visceral branches, at least so far as concerns nerves VII, IX, and X. Sensory branches are spread over gills and adjacent regions exposed to strong stimuli, while branches to the viscera themselves, hidden as they are, are much less open to stimulation. In higher vertebrates the visceral territory of the sensory VII, IX, and X components is relatively less open to powerful stimulation than is the corresponding territory in lower forms, since gills are absent and feeding methods vary. Furthermore, throughout the alimentary tract, sensory terminations are inferior in numbers to motor terminations. Kappers therefore infers that, in the absence of true sensory (reflex) stimulation, the axon type has dominated the system. As an expression of this increasing want of adequate sensory stimulation in the head region of higher forms and the consequent domination of the axon type would come, I should suppose, the development of such structures as the otic, sphenopalatine, and submaxillary ganglia. The factor, according to Kappers, influencing the development of a series of cranial sympathetic ganglia in teleosts, is probably the protecting value of the operculum. In criticism of this largely theoretical work, numerous objections may be raised: first, while it might possibly explain the absence of cranial sympathetic ganglia developed in connection with cranial nerves in general, it could scarcely account for the fact that when sympathetic ganglia do appear, they are developed in connection with the trigeminus—should such be the conclusion eventually reached; secondly, quantitative estimations for relative possibilities of stimulation are little more than suppositions, and Kappers must certainly consider, in this connection, the difference between strength of stimulus and adequacy of stimulus; thirdly, there is

the obvious logical difficulty in arguing from the incompletely demonstrated 'law of neurobiotaxis' to the likewise physiologically undemonstrated 'law of prevalence of the axon-reflex.'

The second group of interpretations, i.e., where it is sought to recognize in certain cerebrospinal ganglia, or in the central nervous system, representatives of sympathetic ganglion cells, has become quite prevalent. Perhaps the most emphatic advocate of this belief is C. K. Hoffmann ('00). Hoffmann's conclusions may be briefly summarized in the following statements:

1. The formation of the sympathetic ganglion in the trunk region is contingent upon the union of posterior and anterior nerve roots to form a mixed trunk.

2. In the majority of head segments this union does not occur.

3. The ramus ophthalmicus V, constituting a purely dorsal sensory nerve, unites with a purely ventral motor nerve, the oculomotor and, as a result of the union, the ciliary ganglion is formed.

4. Since, however, motor and sensory portions of the trigeminus, facialis, glossopharyngeus, and vagus nerves unite within the central organ, they issue therefrom as mixed nerves, and may, therefore, at their origin give rise to sympathetic ganglia. The great peripheral ganglia of these nerves are consequently mixed cerebrospinal and sympathetic, these including jugular, nodose, petrosal, geniculate, sphenopalatine, otic, and submaxillary. The ciliary ganglion alone is purely sympathetic.

The interesting fact in Hoffmann's interpretation is that the single ganglion he has concluded to be entirely sympathetic in nature, the ciliary, is the only one about which serious doubt regarding its single value has definitely existed.¹

¹ Krause ('81) advocated the double nature of the ciliary ganglion. Retzius ('81) described bipolar cells in the ciliary ganglion of the chick, but believed that of the cat purely sympathetic; in 1894 Retzius confirmed his observations. Holtzmann ('96) found bipolar cells in the ciliary ganglion of the frog, the chick, and the dog; he considered the ganglion mixed cerebrospinal and sympathetic. Carpenter ('06) believed that the ciliary ganglion of the chick consisted of two portions, one of bipolar cells and the second of cells typically sympathetic in character. Carpenter's later work (11) failed to confirm the observation. Galvao ('17) described the ciliary ganglion of ophidians as consisting of strictly unipolar cells.

The portion of Hoffmann's explanation most deserving of attention is the part concerned with the possible double nature of those cranial ganglia which have generally been considered similar in structure to the posterior root ganglia of the trunk region. Histological evidence would seem to rest on the recognition of multipolar cells, possibly bipolar cells in the ganglia concerned. Such evidence is apparently scanty (Cajal and Oloriz, '98; Cajal, '06) in the case of the cranial sensory ganglia, but there exists considerable evidence for the presence of multipolar cells in the posterior root ganglia of the spinal nerves. Such, for example, is the contention of Kölliker ('96) (quoting Disse, '93), Lenhossék ('94), Spirlas ('96), Dogiel ('97), Hardesty ('05), and Ranson ('12). Dogiel ('97) examined hundreds of ganglia, finding only occasionally those containing multipolar elements, and then but one, two, or three cells per series. Of the numerous minutely described cell-types indicated in Dogiel ('08), his type XI most nearly approaches our ordinary notion of a sympathetic ganglion cell, but in the absence of any knowledge as to the termination of the so-called 'Hauptfortsatz' and considering the doubtful nature of the 'Dendritenähnliche Fortsätze,' an assignment of these cells to the sympathetic would be premature. Cajal ('06) did not consider bipolar cells found in the vagus ganglion as sympathetic.

Despite the lack of adequate histological evidence, certain morphologists still prefer to interpret the geniculate, petrosal, and nodosal ganglia as in part sympathetic. Such is the conclusion of Hardesty ('14). F. T. Lewis ('13) states that since upon the glossopharyngeal and vagus nerves two ganglia exist—a ganglion of the root and a ganglion petrosum (IX) or nodosum (X), the latter ganglia occupying a position somewhat comparable in relation to the root ganglia as do ganglia of the sympathetic chain to dorsal root ganglia in the spinal region—his tentative attitude is to regard these inferior ganglia (petrosal and nodose) as sympathetic ganglia. Professor Hardesty has been so kind as to furnish me with certain physiological references bearing particularly on the vagus ganglia (Garrey, '12), possibly indicating a vagus influence on the heart independent of cardiac ganglia,

or in other words suggestive of a higher synapse. The reader is referred to the paper in question for discussion. Langley ('03), in discussing the sympathetic action of the vagus, concludes that the ganglion nodosum is not concerned with efferent impulses.

Earlier advocates of the interpretation that some portion of the sympathetic remains behind incorporated into the substance of the cerebrospinal ganglion, may have derived evidence from Gaskell ('86). The latter inferred the presence of non-medullated fibers in the vagus trunk, but failed to find them in the root; his consequent conclusion was that these non-medullated fibers of the vagus trunk were postganglionic fibers arising from small cells of the nodose ganglion. Dogiel ('08), however, described non-medullated axons associated with small cells in spinal ganglia; Cajal ('06) confirmed Dogiel's observations and furthermore showed the axons to be of the T- or Y-shaped variety, and Ranson ('11, '14, and '15) has shown that the axons of these small cells constitute the non-medullated fibers of the peripheral branches of the spinal nerves, and has proved them present both in the trunk and the root of the vagus.

Streeter ('12) suggests that representatives of the sympathetic ganglia of the facialis, glossopharyngeus, and vagus nerves may be present in cells which have remained within the confines of the neural tube or of the sensory ganglion. This possibility is again suggested in connection with the failure of cells of oculomotor origin to contribute to the ciliary ganglion of human embryos.

In certain lower forms there is good evidence for believing that cranial sympathetic ganglia are present in connection with the facialis, glossopharyngeus, and vagus nerves. Anatomical or histological studies show them present at least in teleosts (Stanis, '46, '49, '54; Herrick, '99, '00; His, Jr., '93). Neumayer ('06), in Hertwig's *Handbuch*, states that in selachians cranial sympathetic ganglia other than the ciliary are absent. Johnston ('06) states that the development of cranial sympathetic ganglia reaches so great a magnitude in teleosts that it is to be hoped that it will be discovered in selachians. His, Jr., ('93) found

sympathetic ganglion cells, or small cells which he considered as sympathetic, in connection with the ganglia of the trigeminus, facialis, glossopharyngeus, and vagus in selachians. Similar observations were made in the case of teleostome fishes and Anura. Since His' work appeared before the account of Neumayer, and since it is quoted by Neumayer in other respects, but ignored in this particular, I assume that His' results have not been confirmed. Even the ciliary ganglion seems absent to a large extent in Amphibia. Herrick ('94) found no evidence of a ciliary ganglion in *Amblystoma punctatum*. Coghill states that the ciliary ganglion of *Amblystoma tigrinum* is transitory and probably at no time functional. Norris ('08) reported the absence of a ciliary ganglion in *Amphiuma*, and McKibben ('13) has been unable to discover it in *Necturus*. Kuntz ('14) presents what is perhaps evidence for a transitory ciliary ganglion in *Amblystoma* and for a definitive ciliary ganglion in the frog. Kuntz ('14) reports the presence of both a sphenopalatine and a ciliary ganglion in turtles (*Thalassochelys* and *Chelydra*) and the presence in the chick of four sympathetic ganglia—ciliary, otic, sphenopalatine, and submaxillary.

Although the facts point quite clearly to the presence of sympathetic ganglia in connection with cranial nerves in lower forms, little has been done in determining their origin. His, Jr. ('93) observed the forward growth of the sympathetic chain from the trunk region in the trout and described it as consisting of cell-free fibers having primitively no connection with cranial nerves. Subsequently, connections with trigeminus, glossopharyngeus, and vagus were formed, and at a slightly later stage these connecting strands were found rich in sympathetic ganglion cells. The inference would be that the cells were derived from the respective cranial ganglia. Johnston ('05) states casually that in the teleosts a sympathetic ganglion is present with each cranial ganglion from which same it is presumably derived. Kuntz ('14) has made a detailed study of the development of cranial sympathetic ganglia in the toadfish, but has obtained rather indefinite results.

The impression is gained from Kuntz' work on the toadfish that he regards the cranial sympathetic ganglia of this form as taking origin both from the cranial ganglia and from the neural tube. According to Kuntz, the sympathetic trunk may be traced forward to the Gasserian ganglion in embryos of from 5 to 6 mm. At this early stage the primordia of cranial sympathetic ganglia are to be recognized. The sixth sympathetic ganglion arises in contact with the medial aspect of the first spinal ganglion and of the ganglion jugulare X. Kuntz believes that the ganglion cells of this sixth sympathetic ganglion take origin from the first spinal ganglion, the neural tube, and the jugular ganglion. The fifth sympathetic ganglion arises and perhaps likewise the fourth from cells which have migrated forward from the anlage of the sixth ganglion. The third sympathetic ganglion is derived 'more or less' from the geniculate. The second sympathetic ganglion may be related genetically to both the fifth and seventh nerves, the majority of its cells being doubtless derived from the Gasserian ganglion, and the first sympathetic ganglion is intimately associated with the anterior portion of the Gasserian.

In the turtle Kuntz has suggested a forward extension from the cervical sympathetic ganglion contributing to the formation of both the sphenopalatine ganglion and the otic ganglion. According to this writer, the Gasserian and geniculate ganglia are both associated with the formation of the sphenopalatine ganglion, in the turtle; the geniculate contributes cells to the otic ganglion. The development of the larger cranial sympathetic ganglionic masses in mammals has been followed by Kuntz ('13). Kuntz ('09) has discussed the contribution of the vagus to the sympathetic system. His findings may best be considered somewhat later. Hardesty ('14) states that certain small ganglia of the cephalic sympathetic plexus arise from the semilunar, geniculate, glossopharyngeal, and vagus ganglionic masses.

In no form, apparently, is it clear how much of the cranial sympathetic belongs to the head proper, i.e., with the cranial nerves developmentally, and how much is to be regarded strictly as a forward extension from the cervical or trunk region. Not

until the entire developmental history of the cranial sympathetic ganglia of lower forms has been made a subject for reinvestigation will we be enabled to make any sort of generalization or homology.

MATERIAL AND METHODS

The present study is concerned entirely with the development of the cranial sympathetic ganglia in the mammals. The material used consisted largely of rat embryos. Both albino rats and gray rats were employed, the two series serving to supplement one another. The material available from the departmental collection at the beginning of the work consisted in fifteen series, covering a fairly large range. During the study forty-four series were added to the collection. The latter embryos were obtained from The Wistar Institute of Anatomy and were prepared by the writer with methods especially suited to the type of study demanded. Litters ranging in age from ten and one-half to seventeen days were employed, material being removed at approximately half-day intervals. For general purposes, embryos were fixed in Carnoy's 6-3-1, Zenker's fluid, and Bouin's fluid. In addition silver impregnations were obtained by the Ranson pyridine-silver technique (Ranson, '12). Vom Rath's picro-acetic-osmic-platinic chloride technique, following the procedure of Neal ('14), was used with excellent results in some stages. Where staining was necessary, hematoxylin and orange G. or eosin were generally employed, once Held's erythrosin-methylene blue. Several impregnations of heads of young rats ranging in age from twelve hours to fourteen days were obtained, using the Huber-Guild technique as modified by Larsell ('18). In two cases the glossopharyngeal nerve was dissected out into the tongue and the region removed and stained by the Nissl technique. In order to have some check on the observations of Kuntz ('13), seven pig embryos were examined for the early stages in the formation of the sphenopalatine and otic ganglia. The writer wishes at this point to express his gratitude to Prof. B. F. Kingsbury for originally suggesting the study and for assistance during its progress. Additional expenses incurred

during the investigation have been met through the Mrs. Dean Sage Research Fund. The writer wishes to acknowledge the assistance granted.

OBSERVATIONS

Forward growth of the sympathetic of the cervical region

The first problem for consideration in the study of the origin of the cranial sympathetic ganglia² is that of the forward growth of the sympathetic of the cervical region into the head. Thirteen-day stages in some cases give no evidence of a superior cervical sympathetic ganglion, and therefore, of course, no internal carotid nerve. In others a sympathetic ganglion is present in connection with the first cervical nerve, but no fusion of cervical sympathetic ganglia has occurred as yet. In embryos thirteen and one-half days old, a tendency toward fusion is noted in the cervical sympathetic ganglia, as evinced by the more scattered arrangement of cells and the establishment of a longitudinal connecting strand. In some cases a complete fusion has occurred. Simultaneously with the formation of the longitudinal connecting strand, a forward extension is noted. This anterior extension may be traced forward along the internal carotid artery to a region just dorsal to the pharynx at about the level of the second pharyngeal pouch. The ganglion itself has likewise grown forward to approximately the third-pouch region, whereas in the earlier stage, the most anterior of the cervical sympathetic ganglia lay somewhat caudal to the fourth-pouch region. This more anterior position of the superior cervical sympathetic ganglion seems the result of a large cell increase in

² In this paper the term 'sympathetic' is used in a broad sense to include ganglia of the sympathetic chain, prevertebral ganglia, such as the coeliac, and smaller masses of sympathetic ganglion cells such as are found in the myenteric and submucous plexuses of portions of the alimentary canal and in the various viscera; likewise, the larger ganglionic masses of the head and the so-called ganglionated cephalic plexus (Hardesty, '14). In view of the appointment of a committee, at the 1916 session of the American Association of Anatomists, to examine and revise if necessary the present terminology used in designating these portions of the peripheral nervous system, no more elaborate terminology has been employed by the writer in the present paper.

the interval between the two stages, and is not to be thought of as in the nature of a general forward extension along the internal carotid of cells from the ganglion. The superior cervical sympathetic ganglion ceases abruptly anteriorly; its cells do not 'shade off' among the fibers of the internal carotid nerve.

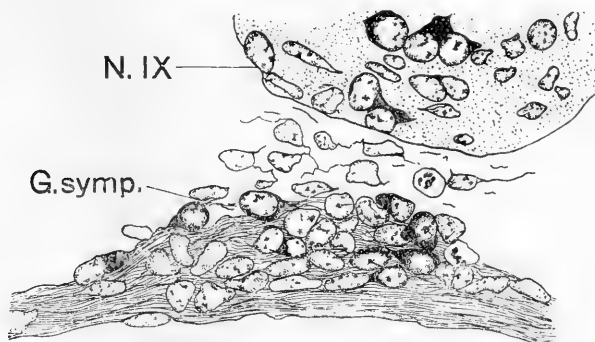
Especially in early stages, complementary pictures are of value. The commoner methods of technique are more useful in demonstrating cell elements than is the pyridine silver. While the latter method has been very effective in demonstrating fibers, it is of only occasional value, in early stages, in furnishing good pictures of the cell bodies. The value of the pyridine-silver technique improves with the increase in cytoplasm of the developing cell body, but even in later stages isolated cell elements tend to be obscured by a wealth of heavily impregnated fibers. At fourteen days little change is indicated, but in fourteen and one-half day stages the pyridine-silver technique gives very excellent pictures of neuroblasts—at this stage unipolar elements—among the fibers of the internal carotid nerve, slightly isolated from the superior cervical sympathetic ganglion. Between this stage and the fifteenth day the outgrowth from the cervical sympathetic ganglion extends sufficiently far forward to join the great superficial petrosal nerve, and from this time on the great petrosal nerve may be definitely recognized. Ganglion cells found in the course of the strands vary greatly in number. In one embryo (pyridine-silver technique) only a single cell was observed between the superior cervical sympathetic ganglion and the point of junction of great superficial petrosal and great deep petrosal to form the Vidian nerve. In others cell masses of considerable size have been encountered, frequently though not always near the origin of the Vidian nerve (fig. 22). The Vidian nerve may be followed into the sphenopalatine ganglion and with it apparently go fibers from the superior cervical sympathetic ganglion. In sixteen-day embryos the internal carotid nerve may be traced as far forward as the carotid canal; ganglion cells are encountered throughout its entire extent. At seventeen days the internal carotid nerve, after giving a branch to the hypophysis, fuses intimately with the abducens; the latter,

soon after, gives a fine anastomotic branch to the trigeminus, and ganglion cells are found along these fibers. These are, needless to say, of scanty number. Koch ('16) and Rhinehart ('18) have reported the presence of sympathetic fibers in the abducens nerve. As regards other outgrowths from the cervical sympathetic, we must consider those fibers passing toward the territory of the middle ear and entering into the formation of the tympanic plexus. They have been given especial attention in view of their later involvement with fibers of the glossopharyngeal nerve, and in view of the possibility of confusing ganglion cells originating from different sources. In fifteen-day embryos strands from the internal carotid nerve, cephalad of the superior cervical sympathetic ganglion, enter the dorsal pharyngeal region and become lost in the mesenchyme over the region of the first pharyngeal pouch. The strands are two in number, and both have been seen to contain ganglion cells, in each case near the origin of the strand. These strands are recognized as the earliest appearance of the superior and inferior caroticotympanic nerves. In sixteen-day embryos the posterior of the two strands has become relatively much larger and numerous ganglion cells are to be noted (fig. 1); seventeen-day embryos show a complete sympathetic anastomosis about the cartilaginous otic capsule. Subsequent to sixteen-day stages there apparently occurs some mixing of glossopharyngeal fibers with fibers from the cervical sympathetic, but the origin of certain ganglion cells of the tympanic plexus apart from any contribution via the glossopharyngeal seems clear. In concluding this section we may state that in addition to the ganglia of the carotid plexus and its extensions, a portion of the ganglia of the tympanic plexus belong with forward extensions from the superior cervical sympathetic ganglion.

Vagus portion of the sympathetic

Coming now to the question of sympathetic ganglia related developmentally to the cranial nerves, it is perhaps most convenient to consider first the vagus group. Previous attempts have been made to show that the vagus plays a part in the

development of sympathetic ganglia. Kuntz ('09) traced the origin of the ganglion cells of the esophageal, gastric, intestinal, pulmonary, and cardiac plexuses to cells which migrated outward from the hindbrain and from the vagus ganglionic masses peripherally along the vagus trunk. From Kuntz' figures, however, I must confess some doubt as to the complete accuracy of his observations. With special techniques and a large series of



1

Fig. 1 Rat embryo, 16 days, Carnoy's 6-3-1. Neuroblasts among fibers of the inferior caroticotympanic nerve. Projection drawing, $\times 500$.

ABBREVIATIONS

<i>A.C.N.IX.</i> , accompanying cells, nerve IX	<i>G.sph.</i> , ganglion sphenopalatinum
<i>B.</i> , bronchus	<i>G.T.E.</i> , tracheo-esophageal ganglion
<i>C.P.</i> , coeliac plexus	<i>Gang. ling.</i> , lingual ganglia
<i>C.S.S.</i> , ganglion cells of cerebro-spinal type	<i>I.</i> , intestine
<i>C.T.</i> , chorda tympani	<i>M.</i> , ramus mandibularis V
<i>E.</i> , esophagus	<i>N.IX.</i> , glossopharyngeal nerve
<i>G.VII.</i> , ganglion geniculi	<i>N.C.I.</i> , nerve cells of the intestinal coat
<i>G.C.</i> , ganglia cardiaca	<i>N.C.R.O.</i> , nerve cells of the ramus ophthalmicus V
<i>G.N.V.</i> , ganglion Gasseri	<i>N.C.N.T.</i> , nerve cells of the nervus terminalis
<i>G.N.X.</i> , ganglion, nerve X	<i>N.R.</i> , nervus recurrens
<i>G.O.</i> , ganglion oticum	<i>N.T.</i> , nervus terminalis
<i>G.p.t.</i> , ganglion of plexus tympanicus	<i>O.</i> , optic cup
<i>G.S.</i> , sympathetic ganglion cells	<i>Ph.</i> , pharynx
<i>G.symp.</i> , ganglion sympathicum	<i>R.P.VII.</i> , ramus palatinus VII
<i>G.S.N.IX.</i> , ganglion sympathicum, nerve IX	<i>R.P.IX.</i> , ramus palatinus IX
<i>G.S.N.X.</i> , ganglion sympathicum, nerve X	<i>Tr.</i> , trachea

embryos, I am free to admit that I have been totally unable to follow Kuntz' 'indifferent cells' back to their ultimate origin, that is to a neural-crest origin as opposed to a neural-tube origin—either one or both. The problem of the origin of the sympathetic ganglia of the trunk region is less difficult. The path of migration is for the most part shorter in the case of the sympathetic ganglia which originate in relation to the spinal nerves than it is for the cranial sympathetic ganglia; one must be exceedingly cautious in accepting statements as to the definite origin of neuroblasts apparently migrating along cranial nerve trunks. We must for the time be satisfied in basing our interpretation largely on future relationships, for at no time apparently does one find a continuity en masse between the sensory ganglion of the vagus and its sympathetic cells such as Held ('09), for example, has figured for the trunk sympathetic. In addition to Kuntz, Streeter ('12) indicates diagrammatically sympathetic neuroblasts migrating peripherally along the vagus into the cardiac plexus.

In thirteen-day rat embryos the vagus has been traced caudad to a point slightly below what has been considered the origin of the superior laryngeal nerve. Within the next half-day the vagus undergoes a rapid caudal extension and may be followed into the condensing mesenchyme about the developing stomach. Small but easily distinguishable neuroblasts are found massed about the growing stump of the nerve and may be traced out into a narrow strand of single cell thickness into the gastric mesenchyme. The first distinguishable phase in the development of the vagus portion of the sympathetic seems, then, the grouping of neuroblasts about the growing tip of the vagus in the lower esophageal region. In addition to these cells, an occasional cell of larger proportions is encountered along the vagus trunk; these elements will be considered in later stages.

For a considerable time the writer was at a loss to account for the rapid increase in the number of neuroblasts along vagus fibers and was inclined to search for large additions from the trunk sympathetic ganglia. Careful search, however, failed to reveal any trace of such contribution, and the true explanation

did not become apparent until later, when the glossopharyngeal nerve was studied. In examining cells in and along the vagus trunk, one finds numerous shapes and sizes which are apparently intermediate between the elongate type of cell and the cell in which cytoplasmic development and general nuclear shape present clearly the characteristics of a neuroblast. These cells possess nuclei showing bendings, constrictions, and tendencies toward the assumption of a spherical shape, apparently correlated with cytoplasmic increase. Vagus fibers are very rich in these elongate elements and their apparent modifications, and it is seemingly through transformation of this indifferent type of cell that the majority of neuroblasts along the vagus trunk arise. In recognizing these elongate cells as elements closely resembling the 'indifferent cells of Schaper' the writer agrees with the interpretation of Kuntz. Only occasionally does one encounter a neuroblast, other than large elements—seemingly aberrant sensory cells—in the upper portion of the vagus trunk; elongate cells and their earlier types of modification are found, however, throughout. The more definitive neuroblastic cells occur lower down, particularly at and below the origin of the inferior laryngeal nerve, in the lower esophageal and upper gastric regions. Here the differentiation process apparently is at a maximum, and in later stages—fifteen-day embryos—large accumulations of neuroblasts may be found along the vagus trunk in the lower esophageal region (fig. 23). This ganglionic mass figured is alone larger than any definitive ganglion of the sympathetic chain at this stage. A similar ganglionic mass has been described by Abel ('09) in a five-day chick embryo. Miss Abel likewise noted similar cells higher up in the vagus trunk, but failed to interpret them of vagus origin; she derived the ganglia of the alimentary canal entirely from the trunk sympathetic ganglia.

Embryos of fifteen and three-quarter days, especially those in the preparation of which special techniques have been employed, have been most useful. It is quite advantageous to compare pyridine-silver preparations, which give excellent fiber pictures, with the results of some excellent cytoplasmic fixer,

such as the v. Rath picro-acetic-osmic-platinic chloride. If we take first the fiber picture alone, we find the superior laryngeal nerve present as a large, well-developed strand. Passing caudad, the vagus gives off fine branches to the developing esophagus, these twigs forming a plexiform mass of fine, often single filaments in the condensing mesenchyme of the future muscularis; no fibers can be traced into the region central to the muscularis. The larger branches anastomose with similar fibers entering from the inferior laryngeal nerve. At the junction of the inferior laryngeal nerve with the vagus trunk there is an accumulation of ganglion cells; their nature will be discussed later when they may be examined to better advantage. Both vagi give off esophageal branches below the level of the bifurcation of the bronchi and send strands into the deeper portion of the cardiac plexus anterior to the bifurcation of the bronchi and above that of the Aa. pulmonales. Plexiform fibers connect the deeper portion of the cardiac plexus with a more superficial portion, with pulmonary branches, and with branches to the lower portion of the vena innominata sinistra. This study does not concern itself with the morphogenesis of the cardiac plexus save as references to it are necessary in general description. The development of the cardiac plexus deserves additional attention; even after my rather superficial examination, it is quite evident to me that the work of His, Jr. ('91), on the development of the cardiac plexus of the chick, needs careful checking. In the abdomen the vagus spreads out over the developing stomach much as in the adult. Just before so doing the left vagus and shortly afterward the right, communicate directly by large branches with the coeliac plexus and indirectly by fine fibers along the gastric branches of the coeliac axis. Due to additions from other sources, above mentioned, it is quite impossible henceforth to determine histologically how far caudad the vagus extends.

Other similarly prepared embryos of fifteen and three-quarter days have furnished quite excellent impregnations of developing neuroblasts in the gastric and intestinal plexuses. Neuroblasts are found in large numbers in the condensing mesenchyme about

the stomach and in still larger quantities (perhaps due to its smaller diameter) in the developing intestinal coat (fig. 2). As regards their origin, the possibility of the addition of elements originating from the trunk sympathetic proper, and migrating outward along the sympathetic branches accompanying the divisions of the coeliac axis, or the superior mesenteric artery, or along direct strands connecting the vagus with the coeliac



Fig. 2 Rat embryo, 15 $\frac{3}{4}$ days. Neuroblasts of intestinal plexus. Projection drawing from pyridine-silver preparation, $\times 500$.

plexus, must be considered. Study of fibers joining the enteric nerve plexus with the coeliac convinces me that any addition from the latter at this stage is small. Later stages have not been examined in search of such additions since the scope of the present paper included merely the demonstration of a ganglion cell contribution of vagus origin. What is perhaps a slight addition from the coeliac ganglionic mass is indicated in the figure to which reference has just been made. In addition, there is

some evidence of an increased number of neuroblasts in the developing intestinal coat at the point where additions from the coeliac ganglion would seem probable. This may, however, be purely physiological (Kappers, numerous papers on neurobiotaxis). It will be noted likewise from the figure that there is some slight indication of a splitting of the enteric plexus into two concentric rings. This division of the plexus is not found continuously; it is scarcely at all evident in the stomach wall, largely so in the upper intestinal tract, and the condition grows progressively less marked in the more caudal loops of the intestine. The doubling of the ring of neuroblasts apparently bears no relation to possible additions from the coeliac. A morphogenetic factor might be suggested as responsible for the late separation into two plexuses—intermuscular and submucous—in the stomach region; for example, the large increase in the lumen of the digestive tube and consequently greatly increased wall surface.

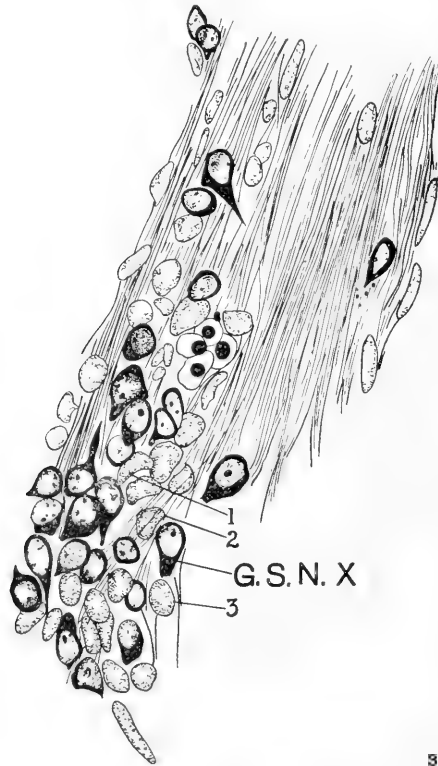
Before discussing in further detail cell types and distribution, it will be necessary to turn to still younger stages in order to determine whether or not neuroblasts are present along the gastro-intestinal tract before any confusion exists between vagus branches and those from the coeliac plexus. Pyridine-silver preparations of fourteen and one-half day embryos show developing neuroblasts in the gastric mesenchyme and in the mesenchyme surrounding the intestine proper. Although nerve fibers are beginning to grow outward along the coeliac axis and superior mesenteric artery, no connection between them and the fibers of the vagus can be made out; they do not enter into gastric or intestinal territory and no cellular contribution to the enteric plexus is indicated. A similar condition has been noted by Kuntz ('09) for pig embryos of from 6 to 7 mm.

When this study was begun it was my belief that all migrant cells—all neuroblasts which had wandered peripheralward from the cerebrospinal ganglionic masses or neural tube (depending on the interpretation assumed)—partook of the nature of sympathetic elements, and I was rather at a loss to account for my ability to quite early classify the neuroblasts into two distinct

types. It was not until some time later when reviewing a paper by Weigner ('05) that the picture became clear. Weigner, in a study of the nervus intermedius, both in man and in the marmot, showed conclusively the presence of T-cells, typical cerebrospinal ganglion cells, on the nervus petrosus superficialis major, the chorda tympani, and especially at the exit of the nerve to the stapedialis muscle. Bipolar cells were likewise encountered. Weigner used material prepared by the Cajal method and there was small chance for error. Rhinehart ('18) mentions a small ganglion at the point where the chorda tympani leaves the facialis trunk, probably of the same nature as the cells described by Weigner, although this is not so stated. This point will be considered later in connection with the facialis.

In the case of the vagus, the two types of peripherally situated neuroblasts are readily distinguishable. One possesses a large, rather clear nucleus and a relatively large amount of deeply staining cytoplasm; the cell seems comparable in every way with elements of the ganglion nodosum; these cells are, in some cases, unipolar, but in the majority of instances, bipolar. In some embryos they are to be found in continuous rows (fig. 24) all the way from the ganglion nodosum to the origin of the inferior laryngeal nerve. I have been able to differentiate them most easily in v. Rath preparations, where the cytoplasm is intensely blackened. The second type of cell, the type which the writer recognizes as a sympathetic neuroblast, is distinguishable by its smaller nucleus, more granular and grayish in its staining capacity, and by the markedly smaller amount of cytoplasm. It seems convenient for future reference to refer to the two cell types as the 'large-cell' and the 'small-cell' type, respectively. The recognition of the small-cell type as sympathetic in nature is based on the following: 1) the cells resemble those elements encountered in the superior cervical sympathetic ganglion and its branches, notably the internal carotid nerve; 2) the cells tend to group themselves in masses, and in these groups the large-cell variety is usually absent; 3) the cells are found particularly in those regions of the vagus trunk where available descriptions of future conditions do not lead us to suspect the presence

of the spinal ganglion cell type; 4) they resemble similar elements in other nerve trunks considered in this study where subsequent observation has shown the cells to be sympathetic, and they are to be found in regions of the vagus where other techniques have indicated sympathetic neuroblasts.



3

Fig. 3 Rat embryo, 15 $\frac{3}{4}$ days, vom Rath's technique. Neuroblasts of vagus trunk, below bifurcation of the bronchi. 1, 2, 3, types of cell, intermediate between elongate accompanying cell and definitive neuroblast. Projection drawing, $\times 500$.

As regards the distribution of the two types, it may be remarked that the large-cell type tends to be found along the main vagus trunk, sometimes, as has been said, in a continuous row from the ganglion nodosum to the origin of the interior laryngeal nerve, at other times more scattered. There is a quite constant aggre-

gation of these cells at the origin of the superior laryngeal nerve and another at the origin of the recurrent nerve. Caudal to the origin of the latter nerve the large cells are but rarely encountered. The small-cell type is of much more general occurrence. They are found in large numbers in and along the vagus trunk (fig. 3); they are likewise found in especially large aggregates at the origin of the recurrent nerve (fig. 4); they are discernible in the cardiac branches of the vagus, in the pulmonary

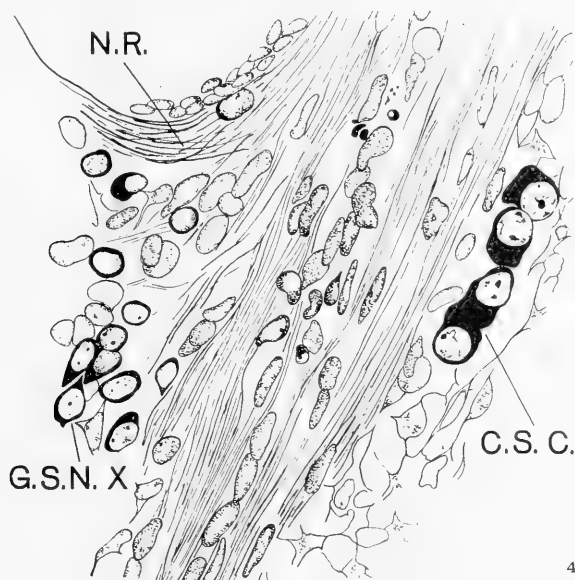


Fig. 4 Rat embryo, 15 $\frac{3}{4}$ days, vom Rath's technique. Neuroblasts of vagus trunk, at origin of N. recurrens. Projection drawing, $\times 500$.

branches, on vagus fibers in the mesenchyme between trachea and esophagus continuous with similar aggregates in the main trunk, and in the gastric and intestinal mesenchyme (figs. 5 and 6). The entire vagus has become in fifteen and three-quarter day embryos the site of neuroblastic differentiation.

Attention may be called at this time to the fact that no such pictures as are indicated in the figures of Kuntz ('09), for the pig, have been obtained in the case of the rat. Although fifteen and

three-quarter day rat embryos are relatively much farther advanced than the 6-mm. pig embryos figured by Kuntz (the period of gestation in the rat averaging but twenty-one days), neither silver impregnations nor v. Rath preparations give any indication of the presence of neuroblasts surrounding the esophagus, as

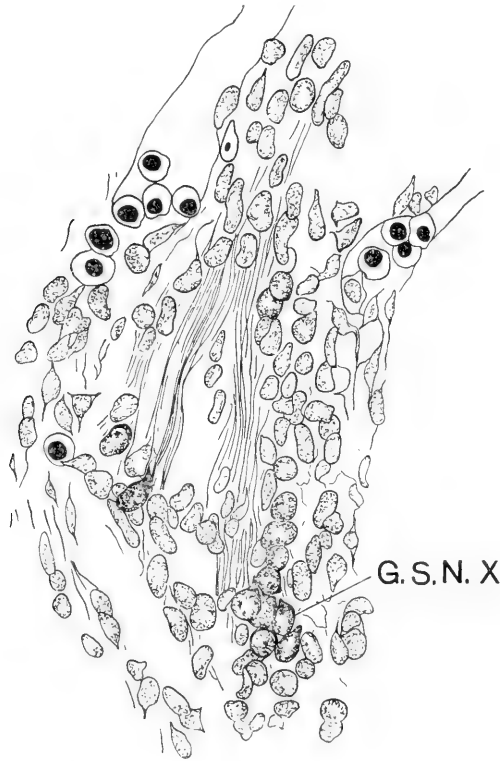


Fig. 5 Rat embryo, 15 $\frac{3}{4}$ days, von. Rath's technique. Neuroblasts along gastric branches of the vagus. Projection drawing, $\times 500$.

the anlage of myenteric and submucous plexuses. The fibers, to be sure, are present, but up to the present nerve cells have not been encountered in any such relation. Considering the excellent impregnations which have been obtained of the corresponding gastric and intestinal plexuses, there is little reason for supposing that the failure to demonstrate them in a peri-esophageal position is due to faulty technique.

An examination of later stages—sixteen- and seventeen-day embryos—affords some few additional facts. The ganglionic mass at the origin of the recurrent nerve has increased somewhat in size. Ganglia on the branches of the inferior laryngeal nerve and on small vagus branches below the exit of this nerve have assumed a constant position as regards the trachea and esophagus. They are situated between the trachea and the esophagus; their number is not constant, averaging perhaps eight per side; as



6

Fig. 6 Rat embryo, 15 $\frac{3}{4}$ days, vom Rath's technique. Neuroblasts along duodenal branches of vagus. Projection drawing, $\times 500$.

one might assume from their origin, they are bilaterally symmetrical. The ganglia tend to be irregular in shape, rather diffuse groups of cells shading into one another; occasionally they are well formed, compact masses. They possess transverse, intercommunicating strands and give off branches to the developing musculature of the trachea. Apparently the occasional neuroblasts which may now be encountered in the developing muscularis of the esophagus and were not found in earlier stages owe their origin to cells found in vagus and inferior laryngeal strands independent of these so-called tracheo-esophageal gan-

glia. These cells together with a tracheo-esophageal ganglion are indicated in figure 7. Below the bifurcation of the trachea, ganglia are found between the bronchi; the most caudal of these fuse with the large masses of ganglion cells constituting the

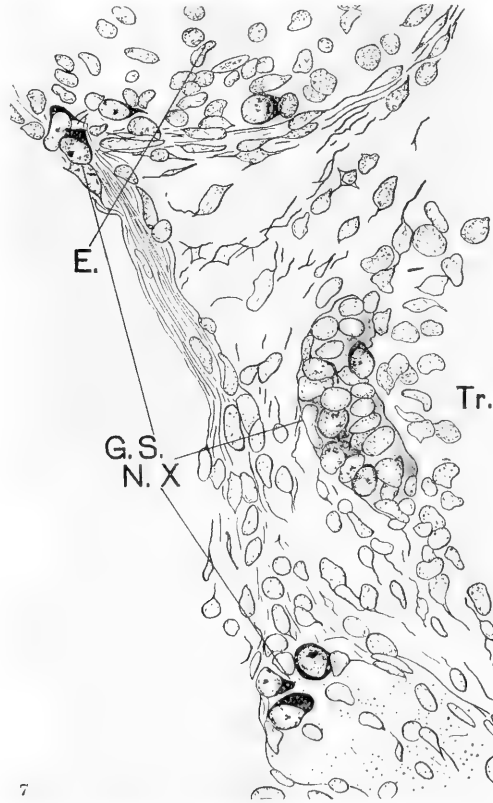
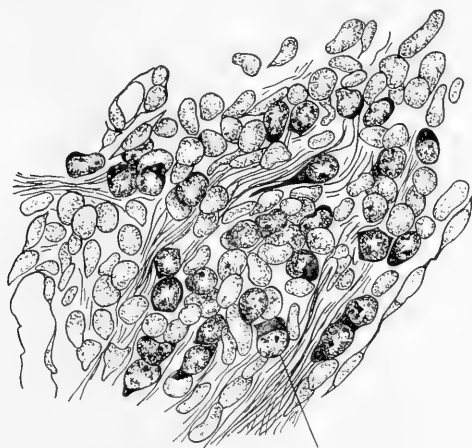


Fig. 7 Rat embryo, 17 days, Carnoy's 6-3-1. Neuroblasts of vagus trunk, a tracheo-esophageal ganglion, and developing ganglion cells of the esophageal plexus. Projection drawing, $\times 500$.

deeper portion of the cardiac plexus. Subsequently this ganglionic mass gives rise to four (two bilaterally symmetrical) offshoots (fig. 25) related apparently to the bronchi and seemingly reminiscent of the same fundamental plan of arrangement as that found higher up. Branches from the vagus proper and from

the cardiac plexus, all rich in ganglion cells, enter the hilus of the lung in association with the bronchi, and the pulmonary artery and vein.

A large number of ganglion cells are encountered along the branches of the superior laryngeal nerve. Certain of these near the vagus trunk are apparently sensory cells continuous with those of the ganglion nodosum. Others (fig. 8) I have considered as sympathetic. Apparently fairly late material is necessary to fully follow the development of the small ganglia of the esopha-



G.S.N. X

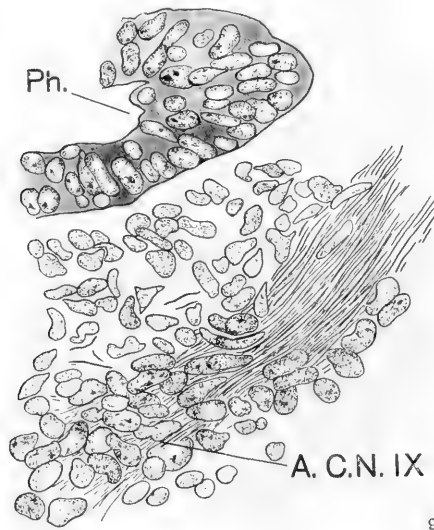
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Fig. 8 Rat embryo, 17 days, Carnoy's 6-3-1. Neuroblasts among fibers of the nervus laryngeus superior. Projection drawing, $\times 500$.

geal and pharyngeal plexuses. The writer hopes to undertake the investigation as time and material become available. Some attention, however, has been paid to the ramus pharyngeus vagi. The ramus pharyngeus vagi is in later stages so closely related to branches from the cervical sympathetic ganglion and to glossopharyngeal branches, that the study of these older embryos is rather unproductive of definite results. In sixteen-day embryos the ramus pharyngeus vagi may be traced fairly separately into the developing muscularis of the pharynx; at this time no neuroblasts have been found among its branches.

Shortly afterward the intermingling of fibers from other nerves so complicates the region that nothing may be ascertained. The ramus pharyngeus vagi is considerably smaller at this stage than the corresponding glossopharyngeal branch. The latter will be considered in a subsequent connection.

In briefly concluding the section on the vagus, the statement may be reiterated that, far from finding that the vagus plays



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Fig. 9 Rat embryo, 15 days, Carnoy's 6-3-1. Elongate cells massed along fibers of the ramus lingualis IX as it enters the tongue. Projection drawing, $\times 500$.

no part in the development of sympathetic nerve cells, the author's results seem to indicate that part if not all of the nerve cells found in the cardiac, gastric, tracheal, esophageal, pulmonary, and upper intestinal plexuses are of vagus origin.

Glossopharyngeal portion of the sympathetic

An examination of the glossopharyngeal nerve for developing sympathetic neuroblasts must include the study of three branches, namely, the lingual branch, the pharyngeal branch, and the

ramus palatinus (ramus tympanicus or Nerve of Jacobson). These divisions will be considered in the order named.

In 5.5-mm. embryos (gray rat) the lingual division of the glossopharyngeus may be traced into the floor of the pharynx as an exceedingly cell-rich strand (fig. 26). It is composed of cells quite closely resembling elements found within the ganglion petrosum, and some difficulty is experienced in distinguishing the exact limits of the ganglion. The contrast between the glossopharyngeal nerve and the hypoglossal, a purely motor



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Fig. 10 Rat embryo, 15 $\frac{3}{4}$ days, vom Rath's technique. Similar cells along finer branches of the ramus lingualis IX in the tongue. Projection drawing, $\times 500$.

nerve, is very marked (fig. 26). The latter nerve is practically free from accompanying cell elements. Slightly later stages (fourteen and one-half and fifteen-day albino rats) show clusterings of elongate cells, similar to those found in the early vagus trunk, along the glossopharyngeal nerve as it enters the tongue (fig. 9), and shortly afterward they are evident in the finer lingual branches of the nerve (fig. 10). As yet no definitive neuroblasts are evident; the writer believes that the definite test for the presence of a developing ganglion cell must be, as in the case of the vagus, the development of the characteristic

cytoplasm. This cytoplasmic increase is little marked in sixteen-day embryos, but the branches of the glossopharyngeus in the tongue show such well-marked clusters of elongate and rounded cell elements (fig. 27) that their neuroblastic character is no longer a matter of doubt. Definitive neuroblasts are found in seventeen-day embryos (fig. 11). They apparently arise, as in the case of the vagus, through a rounding out of the nucleus and through an increase of the cytoplasm in certain of the elongated



Fig. 11 Rat embryo, 17 days, Carnoy's 6-3-1. Neuroblasts along fibers of ramus lingualis IX in tongue. Projection drawing, $\times 500$.

accompanying cells. Rhinehart ('18) has observed sympathetic ganglion cells on fibers of the glossopharyngeal nerve within the tongue. In order to confirm the observations of Rhinehart and to have some check upon my own observations, to establish continuity between the embryonic and the adult condition, four pyridine-silver series through heads of young rats were prepared. In each case it has been possible to locate the constant ganglionic mass described by Rhinehart on the glossopharyngeal nerve near the hyoid bone. It has likewise been discovered in Nissl preparations and the sympathetic character of its cells verified.

In connection with some previous observations of the writer on changes occurring in the foliate papilla of the rabbit after section of the glossopharyngeal nerve, opportunity has existed to observe any changes in ganglion cells of this region (undoubtedly IXth territory) following section. No change and no decrease in ganglion cells has been noted after complete disappearance of taste buds. Circumstantial evidence then would seem to indicate that the general arrangement of these ganglia followed somewhat the scheme of those of the intestinal plexuses, and that they are not entirely dependent on external (cerebrospinal) stimuli. This observation accords with that of Prentiss ('04). The latter noted no changes in cells or fibers (aside from large medullated fibers) in the perivascular and subepithelial networks in the oral epithelium of the frog after section of the palatine branches of the facialis. Prentiss' observation as to the existence of such networks is interesting in connection with the reported absence of cranial sympathetic ganglia—save perhaps a ciliary ganglion—in anura.

The ramus pharyngeus IX, as previously stated, is much larger than the corresponding vagus branch. Although anastomoses with the ramus pharyngeus vagi occur at early stages, I have been able to trace the respective branches quite clearly up to and including sixteen-day stages. At this time certain elongate elements are found in a ganglion-like cluster; a rounding out of the nucleus is to be noted together with the development of a scanty but characteristic cytoplasm, and in seventeen-day embryos large numbers of ganglion cells are to be found in the nerve trunk and among its peripheral terminations (fig. 12); these cells are evident throughout the entire trunk from the ganglion petrosum peripheralward, and one is consequently forced to assume that they originate from migrating cells of the glossopharyngeus. A sympathetic anastomosis is, to be sure, present at this time, but the position of the developing nerve cells of the ramus pharyngeus IX forces one to exclude this anastomosis as a factor in their production.

The adult nervus tympanicus or nervus palatinus IX is quite closely associated with fibers from the internal carotid nerve;

these fibers are ganglionated in later stages. Accordingly, some effort must be made to rule out these neuroblasts migrating from the internal carotid nerve if any contribution along fibers of the glossopharyngeal to the small ganglia of the tympanic plexus is to be shown. If the otic ganglion were of trigeminus origin,



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Fig. 12 Rat embryo, 17 days, Carnoy's 6-3-1. Neuroblasts of ramus pharyngeus IX. Projection drawing, $\times 500$.

it too would have to be considered as a possible source of the neuroblasts of the tympanic plexus, which might then arise through a caudal migration of cells via what in the adult is known as the lesser superficial petrosal nerve. My personal observations have failed to show any developmental relationship between the otic ganglion and the Gasserian ganglion, and I have been forced to the conclusion that the otic ganglion is entirely a ganglion

of the ramus palatinus IX. Kuntz ('13) has derived the otic ganglion of the pig from the Gasserian ganglion and the rhombencephalic wall. Kuntz states that the otic ganglion is well developed before there is any trace of a ramus palatinus IX. In the rat this is certainly not the case. In 5.5-mm. embryos of the gray rat (fig. 13) the first trace of the otic ganglion is to be found as an enlargement in the growing tip of the ramus palatinus IX. In fourteen and one-half day embryos of the albino rat, a large

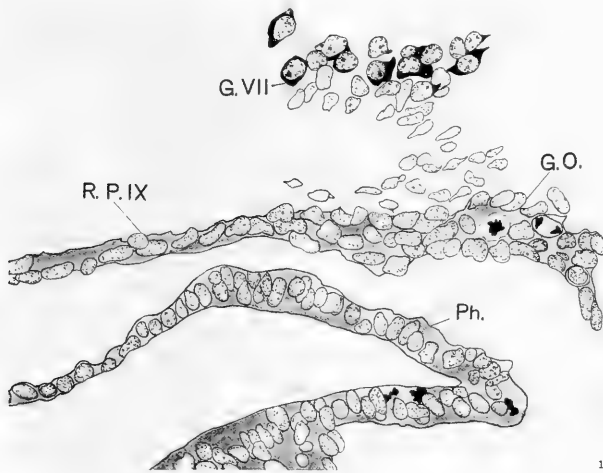


Fig. 13 Rat embryo (gray), 5.5 mm., Zenker's fluid. Anlage of the otic ganglion; marked thickening at tip of ramus palatinus IX. Projection drawing, $\times 333$.

ramus palatinus IX is present, and a few scattered neuroblasts (fig. 14) are present among its terminal fibers. The ramus palatinus IX ends immediately below the ganglion geniculatum VII well posterior to the Gasserian ganglion. No evidence has been found for a migration of cells from the geniculate ganglion into the anlage of the otic ganglion. I am unable to reconcile my observations on pig embryos with the statement of Kuntz. It is difficult to judge when, exactly, he would consider the otic ganglion as 'well established.' Since his first descriptions of the anlage of the otic ganglion are of pig embryos of from 12 to 15

mm. in length, I should scarcely think that at this stage the otic ganglion would be 'well established.' I have found a fairly large ramus palatinus IX in 11-mm. pig embryos and a smaller one in an 8-mm. pig. There seems to be no trace of an otic ganglion, nor have I been able to discover the structure before it was noted as a diffuse collection of cells amid the fibers of the ramus

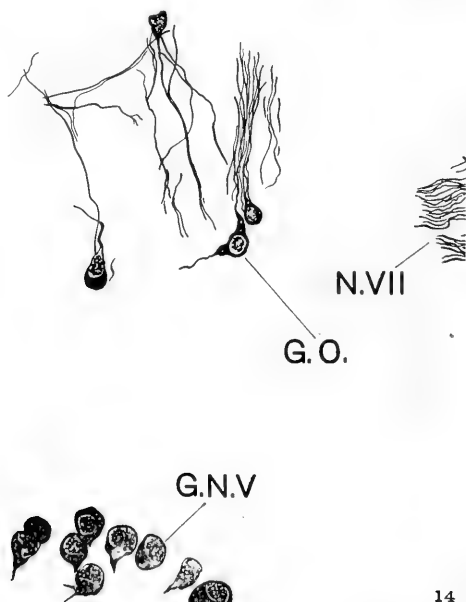


Fig. 14 Rat embryo, 14½ days. Early neuroblasts at tip of ramus palatinus IX. Projection drawing from pyridine-silver preparation, $\times 500$.

palatinus IX, well posterior to the semilunar ganglion and ramus mandibularis V. This is the condition noted in 15- and 16-mm. pig embryos. In 17-mm. embryos the otic ganglion is quite a well-marked structure, but has lost none of the diffuseness which characterizes its earlier stages. The pig embryos which I have examined in this connection form a rather close series (15, 16, 17, 17.5, and 18 mm. total length). The status of the otic ganglion in the 18-mm. pig embryos resembles quite closely that apparent in fifteen-day rat embryos (fig. 28). In the rat, nerve

strands connecting the trigeminus with the otic ganglion are to be noted in sixteen-day embryos. The sympathetic root is likewise apparent. Neither of these have the appearance of migration paths. In the pig, 17.5-mm. stages have shown small aggregates of ganglion cells along the ramus palatinus IX well posterior to the otic ganglion. Although no attempt has been made to follow these in the pig, they are probably to be thought of as belonging to the glossopharyngeal contribution to tympanic plexus.

In sixteen-day rat embryos, the ramus palatinus IX contains numerous neuroblasts throughout its course from the ganglion petrosum to the otic ganglion; some tendency toward the formation of cell aggregates is noted, and in two seventeen-day embryos examined the ganglion cells on the ramus palatinus IX were found to consist of three bilaterally symmetrical groups. The first ganglion appears at the point of origin of the ramus palatinus IX (at this stage slightly distant from the ganglion petrosum); the second (fig. 29) appears at the junction of ramus palatinus IX with the inferior caroticotympanic nerve, and the third at the point of union with the superior caroticotympanic nerve (fig. 30). Were it not for the general distribution of neuroblasts throughout the ramus palatinus in earlier stages, examination of seventeen-day embryos might convey the impression that all of the neuroblasts within the tympanic plexus originated along the caroticotympanic nerves from the superior cervical sympathetic ganglion. In view of this more general distribution, however, the writer is inclined to believe that the cells are of double origin, along the glossopharyngeal and from the superior cervical sympathetic ganglion.

Facialis portion of the sympathetic

Kuntz ('13) has concluded that in the pig the sphenopalatine ganglion arises along the medial surface of the maxillary division of the trigeminus, from cells which wander peripherally from the semilunar ganglion. Streeter ('12) likewise derives the sphenopalatine ganglion from the semilunar ganglion, calling

attention, however, to the closeness of its relation to the great superficial petrosal nerve. Kuntz states that the possibility of a few cells reaching the sphenopalatine ganglion via the great superficial petrosal nerve is not precluded; connection is not made, however, with the latter nerve until the anlage of the sphenopalatine ganglion is well established. On the basis of my own preparations, I have been forced to conclude that the sphenopalatine ganglion is a ganglion of the ramus palatinus VII (the great superficial petrosal nerve). In view of the discrepancy existent in the findings of Kuntz and myself, I have examined

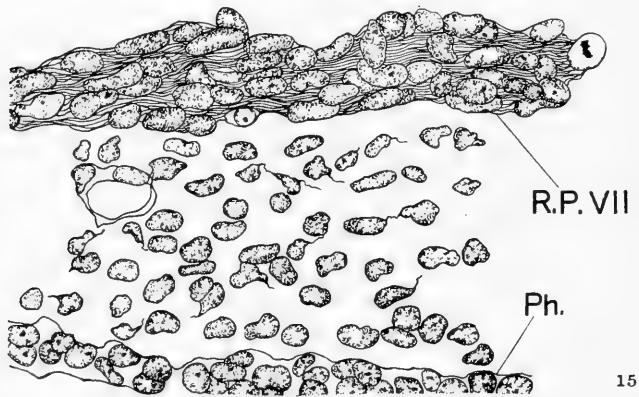


Fig. 15 Rat embryo, (gray), 5.5 mm., Zenker's fluid. A portion of the ramus palatinus VII. Projection drawing, $\times 500$.

a large number of early stages in the formation of the sphenopalatine ganglion, in all twenty-seven series. Of these, seven are of pig embryos of favorable stages. The intensely cellular character of the ramus palatinus VII is apparent in 5.5-mm. rat embryos (fig. 15). The main trunk of the ramus ends anteriorly in cell-rich strands. In fourteen-day rat embryos, the nerve trunk, contrary to the appearance of others, such as the hypoglossus where cell content is apparently restricted to sheath cells, shows toward its growing tip, irregular swellings due to local clusterings of accompanying cells; a considerable extension outward toward the trigeminus is generally noticeable (fig. 31);

the nerve ends anteriorly in a rich, though diffuse, plexus of strands. Similar conditions are approached in 15- and 16-mm. pig embryos. As the number of neuroblasts among the fibers of the ramus palatinus VII is increased, the forward and outward growth of the sphenopalatine ganglion brings it into relation with palatine branches of the maxillary nerve; these branches never, so far as I am able to determine, possess the appearance of a migration path. The palatine branches of the maxillary nerve traverse the sphenopalatine ganglion in fourteen and one-half day rat embryos; the main mass of the ganglion lies posterior to these palatine branches, diffusely scattered along the entering fibers of the ramus palatinus VII. In fifteen-day rat embryos (fig. 32) the sphenopalatine ganglion is an elongated, very diffuse structure, and at slightly later periods, its growth brings it into direct contact with the main trunk of the maxillary division of the trigeminus; with the ganglion well formed, it is difficult to judge whether or not it receives subsequent additions from the semilunar ganglion; so far as I am able to determine, it does not. Still later stages find the sphenopalatine ganglion directly continuous with the semilunar ganglion (fig. 33), with, nevertheless, a clear line of division between their respective cell elements. The development of the sphenopalatine ganglion in the pig is very similar in its early stages (15 to 18 mm.) to that of the rat; later stages have not been followed. It has been traced from a diffuse collection of neuroblasts amid the anterior terminations of fibers of the ramus palatinus VII (figs. 34 and 35) to a relatively compact structure in contact with the main ramus maxillaris V. Its first appearance is well mediad to the palatine branches of the maxillary nerve. In concluding this section on the development of the sphenopalatine ganglion, the fact should be emphasized that in the turtle and in the chick Kuntz ('14) has derived the ganglion from cells migrating outward along the great superficial petrosal nerve. The early diffuse character of the ganglion which he describes in these forms is quite suggestive in view of my own observations on conditions found in the rat and the pig. Certainly, one would suppose that such a structure as the sphenopalatine ganglion would possess fundamentally the same mode of origin in all forms in which it occurs.

Weigner ('05) reported the presence of nerve cells, resembling those of posterior root ganglia, along branches of the chorda tympani and the great superficial petrosal nerve. In the rat I have found them in very small numbers along the chorda (fig. 16), but more regularly along the great superficial petrosal. In addition to an occasional isolated cell, there occurs regularly a small ganglion at the junction of the great superficial petrosal and lesser superficial petrosal nerves; the cells comprising it are typical T-cells. The presence of supposedly sensory cells along fibers of the great superficial petrosal nerve is interesting in view of the assertion of Hoffmann ('00) that the sphenopalatine

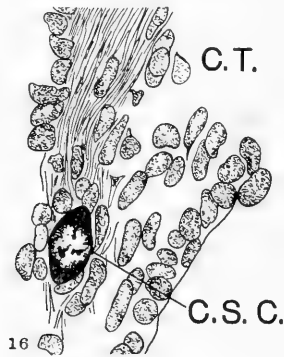


Fig. 16 Rat embryo, 17 days, Carnoy's 6-3-1. Large ganglion cell (cerebro-spinal type) among fibers of the chorda tympani. Projection drawing, $\times 500$.

ganglion was in part sensory. While I have never observed these cells as far anteriorly as the sphenopalatine ganglion, it does not seem impossible that a migration, sufficiently extensive, would bring them to this position. The sphenopalatine ganglion might then occasionally (in some forms) contain typical sensory cells.

In the case of the submaxillary ganglion, I have been unable to substantiate the findings of Kuntz. Kuntz ('13) derives the submaxillary ganglion from cells which wander out from the semilunar ganglion, and from the wall of the rhombencephalon, along the mandibular division of the trigeminus. He finds the ganglion in connection with the lingual nerve before the union of the latter with the chorda tympani. His earliest description

of the ganglion refers to pig embryos of 13- to 16-mm. length. Kuntz' observation on the time of union of the chorda with the lingualis would agree with my own findings in the pig; the two strands have not yet united in an 11-mm. embryo, but from the relative positions of the nerves, the fusion must occur very soon after. In my next available series (15 mm.) the union has occurred and the ganglion is present, at least as closely related to the chorda as it is to the lingualis. In the rat, the submaxillary ganglion does not make its appearance until after the fusion of the chorda and lingualis has occurred. I have been able to examine nine series showing the chorda tympani previous to this period. It resembles closely in structure the early palatine rami (VII and IX) and is proportionally more cellular in character than is the ramus maxillaris. In fourteen-day rat embryos the chorda has united with the lingualis, although at this period the combined trunk is more largely chorda; the submaxillary ganglion has not been found at this stage. Later, when the structure does appear, it lies on that aspect of the combined chorda-lingualis trunk where one would from previous relations recognize mostly chorda fibers. In fifteen-day embryos the ganglion surrounds the combined trunk, but again is mainly on the chorda side; the evidence is of course purely circumstantial, and some account must be taken of the fact that the 'chorda side' of the nerve is likewise that aspect nearest the developing submaxillary gland. Nevertheless, the similarity between the chorda and the ramus lingualis IX and the fact that the more posterior lingual ganglia arise in connection with the latter are strongly suggestive of a chorda origin of the submaxillary ganglion. The greater proportion by far of the small sympathetic ganglia of the tongue are formed as a continuance of the same migration which gives rise to the submaxillary ganglion and the closely allied sublingual ganglion. This formation of small lingual ganglionic masses apparently occurs fairly late; in seventeen-day rat embryos ganglion cells are found in virtually continuous strands, from points on the entering chorda-lingualis trunk into the mesenchyme surrounding submaxillary and sublingual glands, along the submaxillary duct, and upward into the tongue, where they

may be found arranged in a definite row as in figure 36. Rhinehart ('18) has called attention to the presence of ganglion cells along fibers of the hypoglossal nerve in the tongue. Save where fibers of the chorda-lingualis trunk are associated with those of the hypoglossal, no such cells have been encountered in the rat; the main hypoglossal trunk has never been found to contain ganglion cells. Roughly speaking, the ganglia of the anterior two-thirds of the tongue belong with the chorda-lingualis migration, those of the posterior third belong with the lingual division of the glossopharyngeus. Could it be shown that ganglion cells enter the tongue by way of the hypoglossus, it might be most interesting in connection with the transient hypoglossal ganglia described by Prentiss ('10) and others.

Trigeminal portion of the sympathetic

Thus, the possibility of a contribution of trigeminal elements to the submaxillary ganglion cannot be denied, nor have I been able to verify its occurrence. Likewise, I have little to add to the controversy regarding the morphology of the ciliary ganglion. The reader is referred to papers dealing more especially with the ciliary ganglion or with general aspects of cranial nerve morphology for more complete discussion, particularly v. Kupffer ('95), Hoffmann ('96, '97, '99), Johnston ('05), Carpenter ('06), Belogolowy ('10), and the recent paper of Neal ('14). Kuntz ('13) has studied the development of the ciliary ganglion in the pig, and believes it to arise from cells migrating peripheralward from the neural tube along the oculomotor nerve, and from others migrating from the semilunar ganglionic mass along the ophthalmic division of the trigeminus. Streeter ('12) assigns the ciliary ganglion in human embryos to cells which apparently arise from the semilunar ganglion. In this respect my own observations on the development of the ciliary ganglion in the rat accord. The facts to be emphasized are the following: first, the anlage of the ciliary ganglion is to be found in a direct anterior extension over the optic stalk of cells continuous with, and apparently similar to those found in the semilunar ganglion (fig. 17); secondly,

the growth in length of the ramus ophthalmicus V tends toward an isolation of the more anterior elements and a scattering of others along the ramus between its termination and the semilunar ganglion (fig. 18); thirdly, additions to the ciliary ganglion seem to arise from cells which migrate outward along the ophthalmic division of the trigeminus; fourthly, these additions to the ganglion arise as differentiations of elongate cells in the ramus ophthalmicus, and the resulting cell type is constantly smaller than

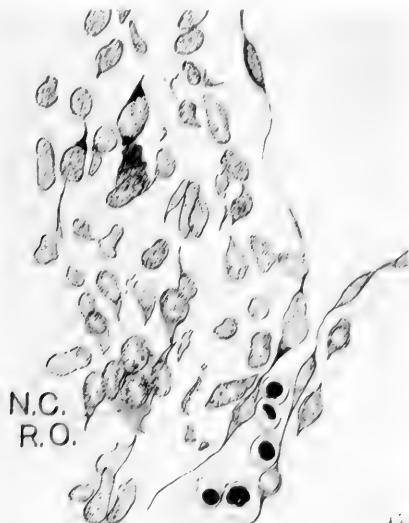


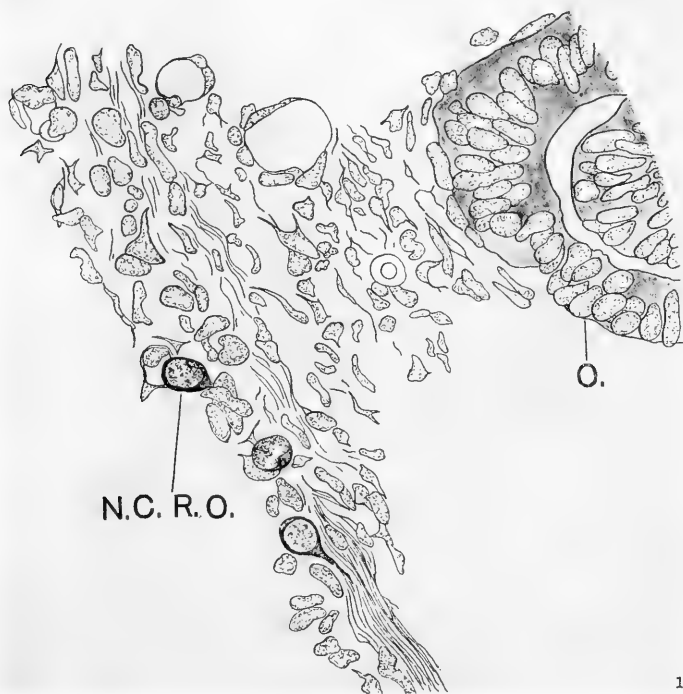
Fig. 17 Rat embryo, 13 days, Carnoy's 634. Neuroblasts in the growing tip of the ramus ophthalmicus V (trans.). Projection drawing, $\times 500$.

the first type observed in the ophthalmic division. The identity of the first type of cell is maintained up until the fifteen and three-quarter stage; after that it has not been distinguished.

The ganglion cells of the nervus terminalis

Realizing that the present tendency of histologists is to regard the ganglion cells of the nervus terminalis as sympathetic in character, some brief attention has been paid their origin. The writer's series is not complete in early stages in the development

of the olfactory sac, and statements made in this regard are admittedly on the basis of limited material. In the rat no evidence of a neural-crest origin for cells of the nervus terminalis, as presumed by Johnston ('09), has been obtained. They, instead, apparently arise from a proliferation of cells of the septal



18

Fig. 18 Rat embryo, 13½ days, Bouin's fluid. Neuroblasts scattered along the ramus ophthalmicus V. Projection drawing, × 500.

aspect of the olfactory sack, including the epithelium of the vomeronasal organ (fig. 19). The possibility suggested by Hardesty ('14), that the ganglion cells of the nervus terminalis may arise as a forward extension from the cervical sympathetic, is scarcely tenable. Cells appear among the fibers of the nervus terminalis (of course, not differentiated from olfactory fila) before there is any forward extension whatever from the cervical sympa-

thetic, and reach large size (fig. 20) much in advance of neuroblasts in the nearest cranial sympathetic ganglion, the sphenopalatine. Despite the apparent sympathetic character of the cells, it must be realized that where the central course of the fibers has been studied, their afferent nature has been strongly

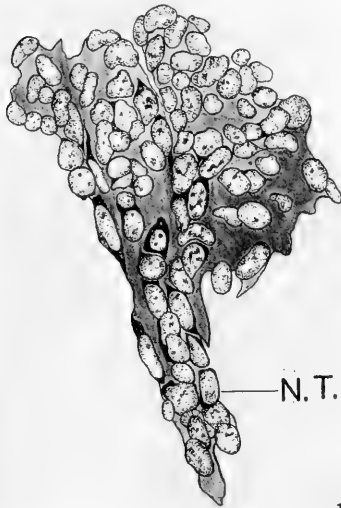


Fig. 19 Rat embryo, 14 days, Carnoy's 6-3-1. Cells of the nervus terminalis, in process of proliferation from the epithelium of the vomeronasal organ. Projection drawing, $\times 500$.

Fig. 20 Rat embryo, 15 $\frac{1}{4}$ days, vom Rath's technique. Neuroblasts among fila olfactoria. Projection drawing, $\times 500$.

suggested, and although many excellent papers on the nervus terminalis exist, no one person has followed both central and peripheral course in a single form.

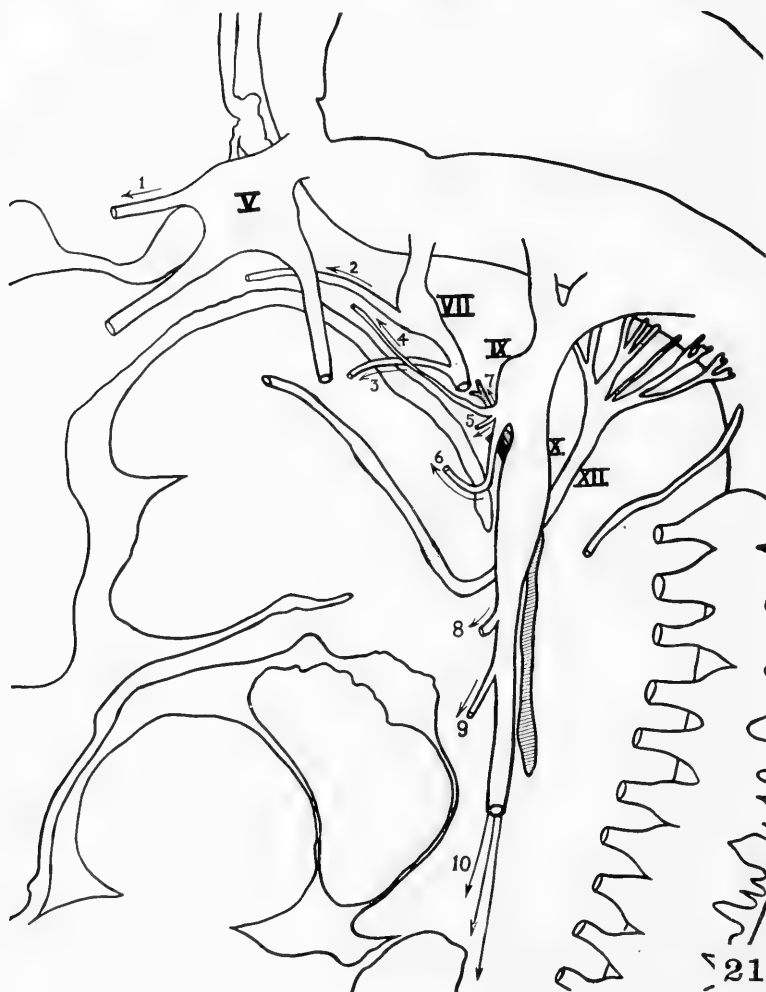


Fig. 21 Schema to illustrate the writer's interpretation of the origin of sympathetic ganglia along the various cranial nerves. 1, The ciliary ganglion. 2, The sphenopalatine ganglion. 3, The submaxillary and sublingual ganglia, together with certain small ganglia of the anterior two-thirds of the tongue. 4, The otic ganglion and certain ganglia of the plexus tympanicus. 5, Pharyngeal ganglia. 6, Small ganglia of the posterior third of the tongue. 7, Ganglia of the internal carotid plexus, its allied plexuses, and certain ganglia of the tympanic plexus. 8, Ganglia along branches of the superior laryngeal nerve. 9, Cardiac ganglia. 10, Esophageal, tracheo-esophageal, gastric, pulmonary, and intestinal ganglia.

SUMMARY

1. Large numbers of cells of vagus origin reach the cardiac, intestinal, gastric, tracheal, esophageal, and possibly pharyngeal plexuses.

2. Cells of glossopharyngeus origin give rise to certain small ganglia of the pharyngeal wall, the posterior third of the tongue, the tympanic plexus, and, in addition to these, to the otic ganglion.

3. The sphenopalatine ganglion is a ganglion belonging developmentally to the ramus palatinus VII (great superficial petrosal).

4. The sphenopalatine and otic ganglia are therefore developed from cells migrating along those nerve trunks which, in the adult, carry preganglionic fibers to the ganglia.

5. Circumstantial evidence favors the interpretation that the submaxillary and sublingual ganglia, together with certain small ganglia of the anterior two-thirds of the tongue, are of facialis origin, the path of migration being the chorda tympani.

6. Neuroblasts giving rise to the ciliary ganglion reach the orbit by way of the ramus ophthalmicus V.

7. The ganglion cells of the nervus terminalis originate in a proliferation of cells of the olfactory sac.

8. Ganglion cells of the carotid plexus and its allied plexuses, together with a portion of the cells of the tympanic plexus, arise as extensions forward from the superior cervical sympathetic ganglion.

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PLATES

ABBREVIATIONS

<i>B.</i> , bronchus	<i>G.S.N.IX</i> , ganglion sympathicum, nerve IX
<i>G.C.</i> , ganglia cardiaca	<i>G.sph.</i> , ganglion sphenopalatinum
<i>Gang. ling.</i> , lingual ganglia	<i>G.T.E.</i> , tracheo-esophageal ganglion
<i>G.N.X.</i> , ganglion, nerve X	<i>M.</i> , ramus mandibularis V
<i>G.O.</i> , ganglion oticum	<i>N.IX.</i> , glossopharyngeal nerve
<i>G.p.t.</i> , ganglion of plexus tympanicus	

PLATE 1

EXPLANATION OF FIGURES

22 Rat embryo, 15 days, Carnoy's 6-3-1. Neuroblasts massed along the internal carotid nerve. Photograph, $\times 105$.

23 Rat embryo, 15 days, Carnoy's 6-3-1. Large aggregate of neuroblasts along vagus trunk, level of cardia. Photograph, $\times 65$.

24 Rat embryo, 15 days, Carnoy's 6-3-1. Ganglion cells continuous with those of the ganglion nodosum, as far caudad as the origin of the inferior laryngeal nerve. Photograph, $\times 50$.

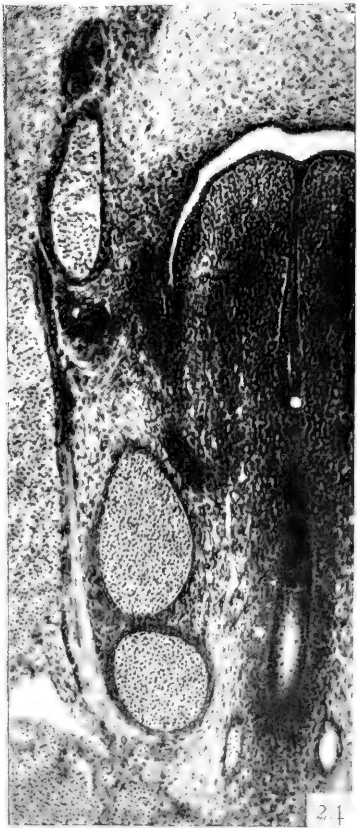


PLATE 2

EXPLANATION OF FIGURES

25 Rat embryo, 17 days, Carnoy's 6-3-1. Continuation of series of tracheo-esophageal ganglia along bronchi, and into cardiac plexus. Photograph, $\times 145$.

26 Rat embryo (gray), 5.5 mm., Zenker's fluid. Ramus lingualis IX. Photograph, $\times 180$.

27 Rat embryo, 16 days, Carnoy's 6-3-1. Clusters of neuroblasts along fibers of ramus lingualis IX in tongue. Photograph, $\times 142$.

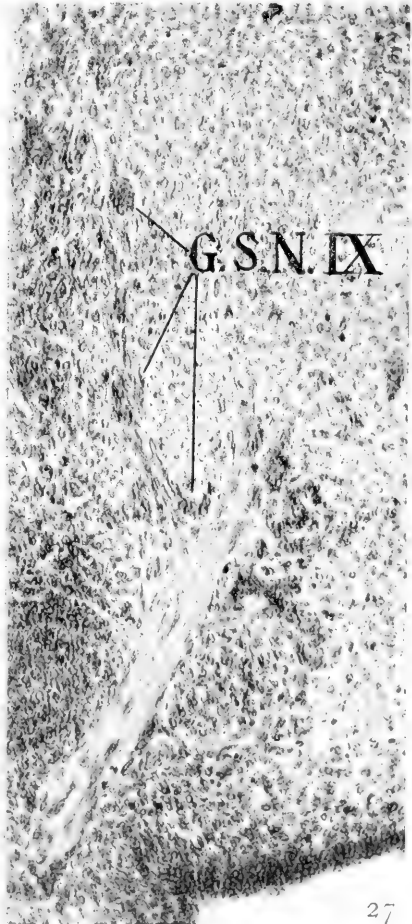
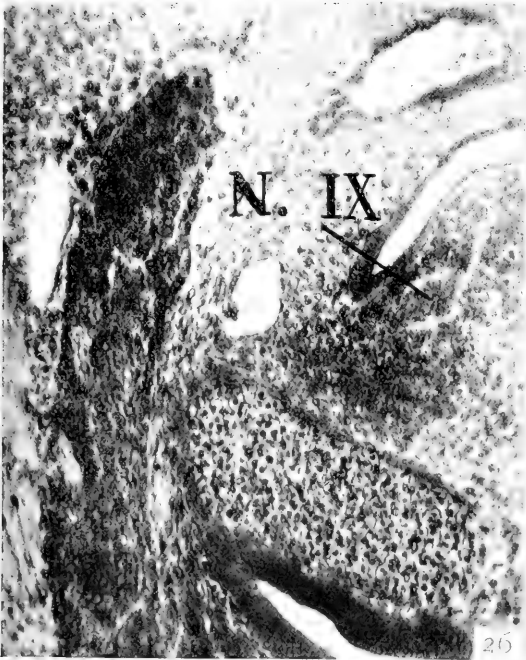
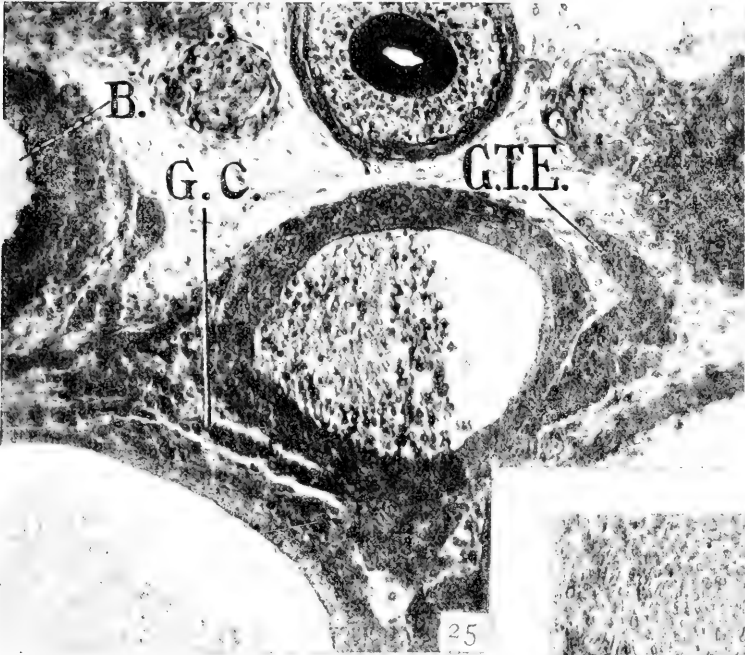


PLATE 3

EXPLANATION OF FIGURES

28 Rat embryo, 15 days, Carnoy's 6-3-1. Gasserian ganglion geniculate ganglion, and otic ganglion. Photograph, $\times 55$.

29 Rat embryo, 17 days, Carnoy's 6-3-1. Ganglion of the plexus tympanicus. Photograph, $\times 130$.

30 Same.

31 Rat embryo, 14 days, Carnoy's 6-3-1. Ramus palatinus VII; a marked lateral extension near its anterior extremity indicates the anlage of the sphenopalatine ganglion. Photograph, $\times 360$.

32 Rat embryo, 15 days, Carnoy's 6-3-1. A still rather diffuse, elongated sphenopalatine ganglion. Photograph, $\times 92$.

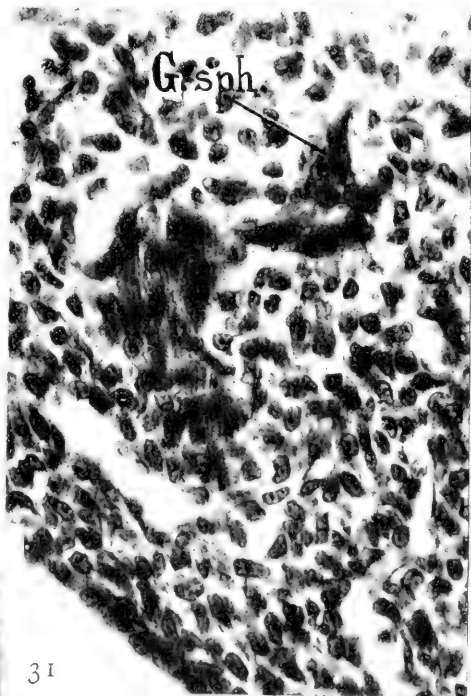
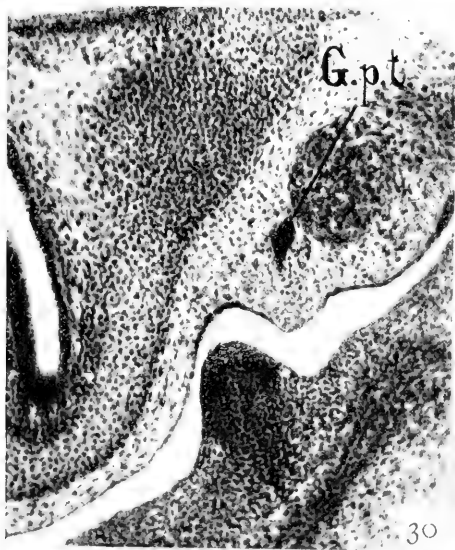
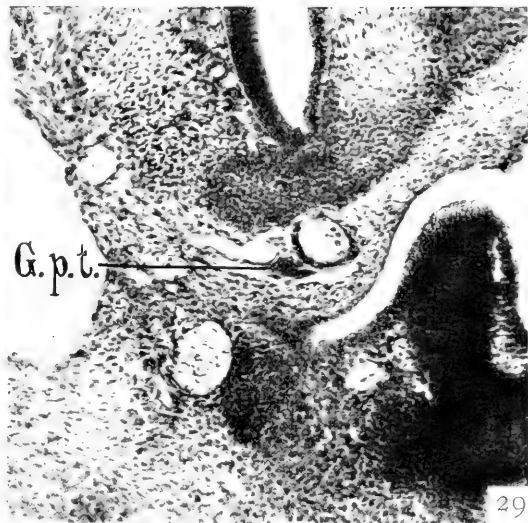
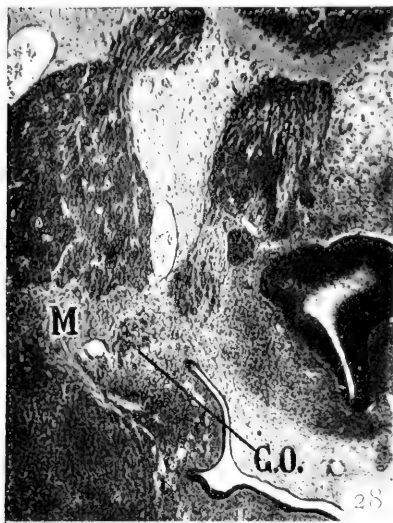


PLATE 4

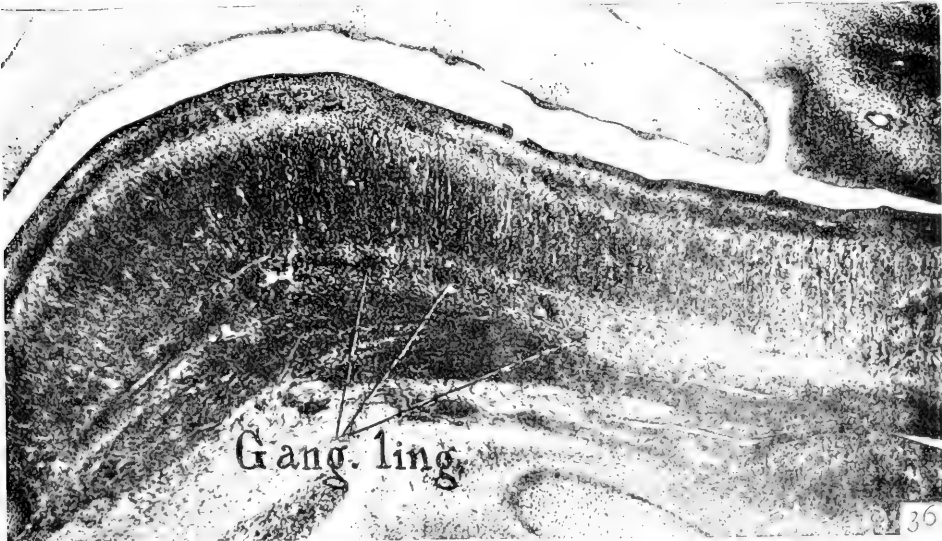
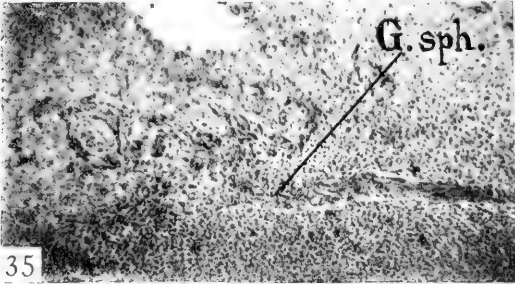
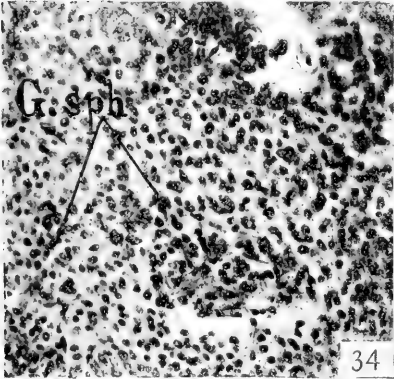
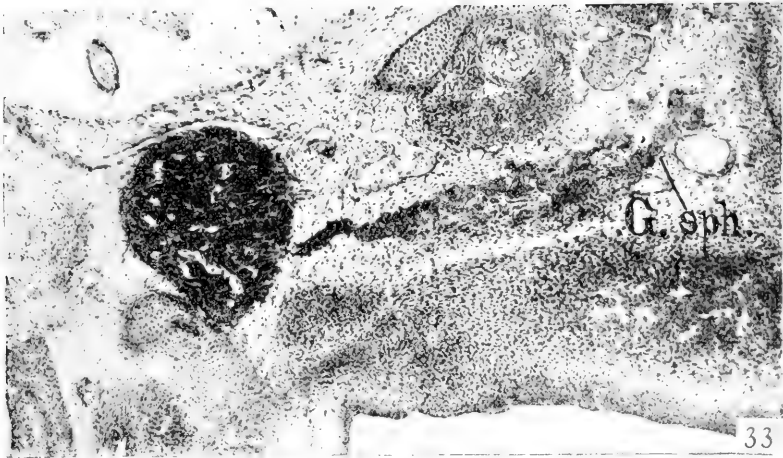
EXPLANATION OF FIGURES

33 Rat embryo, 17 days, Carnoy's 6-3-1. A late stage showing the sphenopalatine ganglion in contact with the Gasserian ganglion. Photograph, $\times 55$.

34 Pig embryo, 15 mm., Zenker's fluid. Diffuse cell strands, extending outward from the ramus palatinus VII. Photograph, $\times 175$.

35 Pig embryo, 17.5 mm., Zenker's fluid. A fairly large but still exceedingly diffuse sphenopalatine ganglion. Photograph, $\times 80$.

36 Rat embryo, 17 days, Carnoy's 6-3-1. Developing lingual ganglia. Photograph, $\times 55$.



Resumen por el autor, Henry Carrol Tracy.
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El laberinto membranoso y su relación con el divertículo precelómico de la vejiga natatoria en los cupleoideos.

En los peces cupleoideos existe alrededor del laberinto y el cerebro un sistema de canales perilaberínticos formados a expensas de tejidos condensados y espacios intercelulares diferenciados en el tejido perimeníngeo. Los cambios en la presión hidrostática pueden transmitirse directamente a la endolinfa mediante este sistema de canales. El piso del receso utricular está unido a los bordes de la ventana existente en la cápsula del hueso proótico, la cual contiene a la vesícula membranosa de paredes delgadas de la vejiga natatoria. La mancha del receso utricular está subdividida en tres partes, de las cuales la anterior está situada a lo largo de la línea de inserción del piso utricular en el borde fenestral anterior, mientras que la porción media está colocada a lo largo de la inserción en el borde posterior. Entre estas dos porciones maculares se extiende la membrana otolítica a través de la endolinfa. Esta membrana presenta una estructura multilocular, en cuyos espacios se proyectan las pestañas de las células maculares; se desarrolla probablemente a expensas de la cutícula situada sobre dichas células a la cual se une la secreción producida por estos últimos elementos. Las células maculares probablemente forman un receptor estimulado por cambios en la presión hidrostática. Estos cambios se transmiten por los canales perilaberínticos hasta la endolinfa y desde esta por la ventana (estimulando de este modo a las células de la mácula) a la vesícula membranosa de la vejiga natatoria. La estimulación de las células maculares probablemente produce reflejos que rigen el mecanismo regulador de los gases contenidos en la vejiga natatoria o, produciendo movimientos compensadores en la natación, tiende a mantener al pez cerca de ciertos niveles del agua.

THE MEMBRANOUS LABYRINTH AND ITS RELATION TO THE PRECOELOMIC DIVERTICULUM OF THE SWIMBLADDER IN CLUPEOIDS

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INTRODUCTION

In a recent paper (Tracy, '20) I have described the Clupeoid skull and cranial nerves from the point of view of their relations to the precoelomic diverticulum of the swimbladder. In that paper is also included a summary of previous investigations on the ear-swimbladder relation in Clupeoids. This present paper is concerned primarily with the swimbladder diverticulum and the membranous labyrinth and their relations to each other and to the skull. Of particular interest is the macula acustica utriculi, which has not been previously described and which in Clupeoids is different in structure and relations from that in other groups of animals. The probable physiological significance of these structures is also discussed in this paper.

The drawings were made by Mr. Leo Massopust, the department artist at Marquette School of Medicine.

MATERIAL AND METHODS

Adult specimens of the following common American species of Clupeoids were examined: *Alosa sapidissima* (shad), *Pomolobus pseudoharengus* (alewife), *Pomolobus aestivalis* (summer herring), *Pomolobus mediocris* (hickory shad, fall herring), *Brevoortia tyrannus* (menhaden). The shad specimens were bought in local markets; specimens of the other species were obtained from the Marine Biological Laboratory where they had been preserved in 10 per cent formalin.

In addition to the ordinary dissection methods, decalcified heads of *Pomolobus pseudoharengus* were embedded in celloidin and cut in various planes in sections $50\ \mu$ in thickness. This method was particularly useful in the study of the condensed, perilabyrinthine tissue and the canals contained within it. For the study of these structures by dissection, *A. sapidissima* is the most favorable of these species, because the perilabyrinthine structures are larger and better defined than in the smaller species. For the demonstration of the perilabyrinthine spaces, some use was made of the cedar oil and chloroform method (Locy, '16, p. 542).

In addition to adult specimens of the above-named species, there were also available a few young specimens of *Brevoortia tyrannus* and about thirty stages of *Stolephorus mitchilli* (anchovy) ranging from 3 mm. up to half-grown specimens. These were sectioned in various planes and stained by standard methods. Most of these specimens I obtained some years ago from the lobster rearing cars at the Experiment Station of the Rhode Island Commission of Inland Fisheries, then under the directorship of Dr. A. D. Mead, of Brown University.

TERMINOLOGY

The remarkable series of spaces and differentiated tissues which are described in detail in the body of this paper are undoubtedly modifications of the perimeningeal tissue which pervades the intracranial region outside the meninx primitiva. In discussing them, it is not easy to select terms which have an

accurate morphological value. The term 'perimeningeal' must be retained for the undifferentiated tissue around the brain and labyrinth, and cannot, therefore, with definiteness be applied to structures differentiated from it. The term 'perilymph' or 'perilymphatic' which has been used by previous writers in this connection is subject to the same objections which Streeter ('17) has advanced in discussing the somewhat analogous structures around the membranous labyrinth in higher vertebrates.

The term 'periotic,' or 'perioticular,' as it has been applied to the tissues and spaces around the membranous labyrinth of higher vertebrates is also objectionable in the case of the Clupeoids. Only a small part of the labyrinth in these fishes is enclosed in an auditory capsule. The tissues and spaces around the labyrinth are, therefore, continuous with, and never differentiated from, the corresponding structures around the brain, but they form a part of an extensive system of differentiated tissues and spaces which extend through the perimeningeal tissue both above and below the brain and which reach out through the lateral recess of the skull to the lateral-line canals. These relations are obviously different in many respects from those of the spaces around the membranous labyrinth of higher forms to which Streeter applied the term 'periotic' or 'perioticular.' The use of this term in the case of the Clupeoids implies an homology which is certainly not complete and which probably has little, if any, morphological significance.

The term 'perilabyrinthine' has therefore been chosen to apply to all the specialized, condensed tissue and the spaces in it, which are developed around the membranous labyrinth and their extensions over and under the brain. 'Perimeningeal' will be used as is customary (following Sterzi) to designate all the undifferentiated tissue between the meninx primitiva and the cranial wall. It may be noted here that in certain regions of the Clupeoid skull this tissue extends through large apertures in the cranium and becomes continuous with the subcutaneous tissue, particularly in the neighborhood of certain parts of the lateral-line canals. Here accurate use of terms may be sometimes diffi-

cult, but perimeningeal will apply to all this tissue except that which is obviously subcutaneous.

The term 'marginal membrane' (used by Streeter for the membrane limiting the developing periotic spaces of the human embryo) seems appropriate for the incomplete connective-tissue walls found around some of these spaces.

THE GENERAL HISTOLOGICAL STRUCTURE OF THE SWIMBLADDER AND THE PRECOELOMIC DIVERTICULUM

The wall of the swimbladder is composed of a lining mucosa (tunica interna) and an outer strong connective-tissue layer (tunica externa). A thin layer of loose connective tissue (submucosa) joins these two layers. The histological details of these layers in the Clupeoids have not been investigated completely. The epithelium in most parts of the organ is simple squamous; in some places, however, and particularly in the pneumatic duct, it is cuboidal or columnar, and in *Alosa sapidissima* is thrown up in complicated folds which possibly form a primitive type of 'red gland.' The tunica propria of the mucosa seems to consist of a delicate connective or reticular tissue, with a network of fine elastic fibers in a layer under the epithelium; in the outer part of the mucosa is a second layer of elastic tissue. In *Stolephorus* a thick band of circular smooth muscle occurs in the tunica propria at the constriction between the two parts of the swimbladder, in the walls of the pneumatic duct, and at the entrance of the precoelomic diverticulum into the cartilage tube.

The tunica externa is much thinner in the Clupeoid swimbladder than in that of many other teleosts. It is composed almost wholly of coarse bands of connective tissue running circularly in a compact layer around the organ. The only elastic elements shown in my sections are fine fibers running in a radial direction through this layer.

The precoelomic diverticulum of the swimbladder is formed in the embryo by an anterior outgrowth from the forward end of the anlage of the primitive swimbladder which pushes cephalad into the cranial cavity. As might be expected from the manner

of its development, the diverticulum is structurally a continuation of the mucosa (tunica interna) of the swimbladder, and is made up of corresponding layers. The epithelium of the swimbladder continues from its anterior end (here simple squamous) into the diverticulum, where it becomes columnar and is thrown up into simple folds which suggest a glandular structure (fig. 1). In the parts of the diverticulum inside the bones of the skull, the epithelium becomes low cuboidal or squamous and is not thrown up in folds (Stolephorus and Pomolobus). The epithelium of the membranous vesicles is simple squamous (fig. 17). The tunica propria in the tubular part of the diverticulum is thin but dense and at the beginning of the diverticulum in Stolephorus contains a layer of circular smooth muscle. Very loose, sparse connective tissue (submucosa) suspends the tube in the cartilage canal (fig. 1). The connective tissue attaching the membranous vesicles to the inner surface of the osseous capsules is very loose; here the tunica propria and submucosa blend into each other.

The posterior membranous vesicle of the swimbladder diverticulum, which lies in the posterior osseous capsule, is practically the same in structure as that of the anterior membranous vesicle. Since the posterior vesicle has no apparent relation to the labyrinth or other structure outside the bony capsule, it will not be discussed further in this paper.¹

The cartilage tube around the diverticulum is continuous with the tunica externa of the swimbladder and is probably the morphological representative of that layer. The cartilage in Stolephorus and Pomolobus presents, in sections, a somewhat peculiar microscopic appearance, since the cartilage cells are larger than usual in comparison with the amount of intercellular

¹ The development of this vesicle is almost unknown. It is not formed until comparatively late stages. It is not present in Stolephorus or Brevoortia in specimens earlier than 30 mm. in length. It is, however, fairly well developed in a young Stolephorus 38 mm. in length. The relations shown in this specimen indicate that it develops by the formation of an outgrowth from the main branch of the precoelomic diverticulum. This outgrowth pushes up into the space within the canal for the diverticulum in the otic capsule (indicated by the end of the reference line *CSD* in fig. 15).

matrix. A peculiarity of the tube in the shad (fig. 2) is the indefiniteness of the perichondrium; the transition from fibrous tissue to cartilage matrix is gradual and matrix formation has proceeded into the surrounding connective tissue in a very irregular manner. There is therefore an intimate connection dorsally and at the sides of the tube with the muscular aponeurosis on which it lies. These conditions produce the roughness which is characteristic of the surface of the tube as seen in dissection. It is attached to the aponeurosis by strong threads of connective tissue. The dimensions of this tube in one adult specimen of *Alosa*, measured from the section by micrometer methods, is as follows: outside diameter, 0.35 mm. in one dimension, 0.6 mm. in the other; least inside diameter, 0.12 mm.; greatest inside diameter, 0.19 mm.; diameter of the membranous diverticulum, 0.05 mm. In *Stolephorus*, outside diameter, 0.14 mm.; inside diameter, 0.075 mm.; diameter of the diverticulum, 0.03 mm.

THE PERIMENINGEAL TISSUE AND THE PERILABYRINTHINE SPACES

The perimeningeal tissue over the top and sides of the brain is for the most part undifferentiated and consists of a layer of connective tissue, thick and fatty in most places, but membranous and fascia-like over the lateral cartilage plate (*Pomolobus pseudoharengus*). This fatty tissue also extends through the temporal foramen and encloses the large bay of the lateral-line canal which lies therein. The perimeningeal tissue extends around the semicircular canals and between the outer side of the sacculus and the corresponding bony wall of the auditory recess; it fills the lateral recess and surrounds the expanded part of the lateral-line canal in the recess. In these latter situations, however, it apparently does not contain fat, but is of a very loose, reticular-like structure.

Immediately around the membranous labyrinth and under the brain, a considerable part of the perimeningeal layer is differentiated into a tissue which is similar to that forming the tunica propria of the epithelium of the labyrinth in other ani-

mals. This differentiated tissue is compact, elastic, tough, and translucent, and is apparently composed of flattened bands, or sometimes of irregular plates of fibro-elastic tissue so closely held together by a cement substance that in sections it appears in most places almost as homogeneous as the matrix of cartilage. Flattened, stellate cells occupy irregular spaces between the fibrous elements.

In the Clupeoids, this differentiated tissue is not confined to the cells and fibers immediately adjoining the epithelium of the labyrinth, but forms definite processes and extensions which reach into remote parts of the perimeningeal tissue. These are the structures referred to as perilabyrinthine plates or processes to distinguish them from the undifferentiated portions of the perimeningeal tissue.

The most extensive of these perilabyrinthine structures involves the greater part of the perimeningeal layer under the brain. It takes the form of a horizontal plate (subcerebral perilabyrinthine plate) which connects the thickened walls of the utriculi of the two sides under the brain (figs. 8 and 11, *SPP*). Anteriorly it extends a short distance in front of the utriculus and its edge curves downward to become attached to the periosteum on the surface of the anterior bony capsules of the two sides and the ridge of bone connecting them (fig. 12, *SPP*). The lateral edge of this forward extension of the plate in front of the utriculus is attached to the arch leading to the lateral recess of the skull (i.e., to the falciform process of the prootic bone); over the utriculus, the lateral part of the plate is continuous with a sharp ridge-like thickening of the differentiated perilabyrinthine tissue of the utricular roof which is continuous with the thin perimeningeal tissue which lines the cartilage in the lateral wall of cranium (fig. 11,*). Posteriorly the plate is attached to the free anterior edges of the triangular plates of the two exoccipital bones, so as to roof in the saccular recess on each side. Thus the plate, with the connected thickening of the utricular roof, forms a 'false bottom,' so to speak, of the cerebral cavity, and by its continuous attachment with the margins of the auditory recess completely excludes the latter from direct

connection with the space around the brain (fig. 8). Over the brain there is also a perilabyrinthine structure in the form of a hollow rod or band which arches over the medulla behind the cerebellum and connects the base of the superior sinus of the labyrinth with the corresponding structure of the other side (figs. 12 and 16, *PSC*).

Less extensive developments of the perilabyrinthine tissue are found in other places, particularly around the superior sinus and the superior end of the anterior semicircular canal (*Alosa*) where it lies free in the cerebral cavity (figs. 7 and 12). There is also a plate of this tissue which begins in the roof of the extreme anterior end of the sacculus and passes laterally involving the ventral wall of the utriculus; it is somewhat triangular in form and extends under the pterotic segment of the arch which leads into the lateral recess (figs. 9 and 12, *TPP*). This plate is probably the structure which Breschet called the 'bulbe accessoire;' Ridewood describes an expansion of the utriculus in this position. Tysowski, however, describes it as merely a three-sided pyramidal thickening of the utricular wall. A reconstruction of the cavity of the labyrinth definitely proved that this structure contains no diverticulum of the labyrinth.

Condensed tissue also occurs as a local thickening of the outer wall of the recessus utriculi and under the divisions of the maculi acustica utriculi. It is important to note that the floor of the recessus utriculi between, and on each side of, the anterior and middle division of the macula acustica utriculi is thin and contains little or no dense tissue (fig. 17).

Through certain parts of these plates and processes of compact perilabyrinthine tissue, the forms and relations of which have just been described, and through certain parts of the undifferentiated perimeningeal tissue is an extensive system of spaces and canals which result from a rarefaction or thinning out of the tissue elements. In the manner of their development, these spaces and canals are probably comparable with the perioticular spaces of the mammalian ear as described by Streeter ('17). In some of these spaces the rarefaction of the tissue is merely relative as compared with the compact tissue; in others

only a few delicate fibers remain in them. These spaces are merely tissue spaces formed by modification of the perimeningeal or perilabyrinthine tissue, and they are probably not directly homologous with the spaces around the labyrinth in higher forms.

These canals are in direct connection with tissue spaces in the lateral recess. The tissue of this cavity is composed of a meshwork or reticulum of connective-tissue strands or trabeculae. It contains no fat. The spaces of this meshwork are of varying sizes and communicate freely with each other. One very large space (in diameter nearly half of the whole width of the recess) appeared in all specimens which I sectioned and is probably constant. It lies close to the lateral-line canal. It is bounded by a very definite marginal membrane of modified connective tissue. Numerous small deficiencies in the membrane, mostly on the mesial side of the space, allow free communication with the spaces in the reticular network. These spaces in turn communicate with the perilabyrinthine spaces mentioned above and, together with them, form a complicated system of intercommunicating canals which have a definite relation with the membranous labyrinth and other structures.

These perilabyrinthine spaces and canals may be considered in three groups, viz., lateral, subcerebral, and supracerebral.

The lateral group of spaces consists of the tissue spaces of the lateral recess which have been described above. They are immediately continuous (figs. 11 and 12) with the utricular subcerebral canal under the recessus utriculi. They also communicate with the supracerebral canal through an opening in the triangular plate (*TPP*) and the reticular spaces which lie lateral to the utriculus and the superior sinus.

The subcerebral group comprises two canals, the utricular subcerebral canal and the saccular subcerebral canal. These canals are excavated from the lower surface of the subcerebral perilabyrinthine plate (fig. 11, *USC* and *SSC*). The lumen of both is occupied by an exceedingly sparse network of very delicate connective-tissue fibers. The utricular subcerebral canal is the more anterior and runs transversely across the floor of

the skull between the two lateral recesses. It begins on each side under the utriculus where it occupies the whole space between the floor of that structure above and the surface of the anterior osseous capsule below (fig. 12). It is partially subdivided into an anterior and posterior part by the projecting lips of the fenestra on which the utriculus rests (figs. 5, 9, 12, and 17; *USCA*, *USCP*). In the utricular region the roof of the canal is formed by the floor of the recessus utriculi in which are located the divisions of the macula acustica utriculi. The floor of the utriculus is very thin except under the divisions of the macula.

Laterally the canal connects freely with the tissue spaces in the lateral recess under the arch made by the apposition of the falciform process of the prootic bone with the projecting anterior part of the pterotic bone. Thus, the lateral spaces of each side communicate freely with each other through the canal. There appears to be no obstacle to the free transmission of pressure changes from the outside through the lateral-line canal and the lateral spaces to the two parts of the utricular subcerebral canal and thence to the floor of the utriculus on each side of the lips of the fenestra.

A small blind extension of this canal passes up over the mesial narrow side of the recessus utriculi in close relation to the epithelium. From the space between the utriculus and the anterior osseous capsule, the canal passes medially straight across the floor of the skull under the brain to become continuous with the corresponding structure of the other side (fig. 11).

The utricular subcerebral canal is the best-defined of all the perimeningeal spaces. It is easily visible to the naked eye and has been observed by all investigators from Weber on. Weber and Ridewood considered it an endolymphatic canal. I have studied many series of sections of young and adult Clupeoids, and there can be no doubt whatever that it is a tissue space, differentiated from the perimeningeal tissue under the membranous labyrinth and brain. Tysowski and de Beaufort, the only investigators to study the canal with modern technic, emphatically testify to the same conviction.

A similar canal short but of larger diameter, connects the loose tissue on the mesial surfaces of the two sacculi. This saccular subcerebral canal has previously been mentioned only by Tysowski.

The supracerebral canal is a space in the hollow band of compact tissue which arches over the medulla behind the cerebellum (fig. 16). As this passes down posterior to the superior sinus of the utriculus, it divides (figs. 4, 5, 6, and 7; *PSCM*, *PSCL*); one branch passes through the compact perilabyrinthine tissue mesial to the sinus and connects with spaces mesial to the sacculus, the other gradually passes behind and then lateral to the sinus and connects with the lateral spaces above mentioned. This branching of the supracerebral canal may perhaps explain the conflicting views of Breschet and Hasse. Breschet must have seen merely that part of the supracerebral canal which passes between the two superior sinuses; Hasse doubtless traced the branch of the canal which passes down to the sacculus mesial to the sinus and assumed it to be the endolymphatic duct.

The upper chamber of the anterior osseous capsule contains only a very sparse connective tissue. It is apparently very similar in structure to the perilabyrinthine spaces. It will be observed, therefore, that only a very loose and rarefied connective tissue intervenes between the elastic septum of the osseous capsule and the thin part of the utricular wall which bridges the fenestra and connects the anterior and middle divisions of the macula.

THE MEMBRANOUS LABYRINTH

The form and structure of the membranous labyrinth of the Clupeoids appear on dissection quite different from those of other teleosts, because the thickenings of the compact tissue, as described above, do not conform externally to the shape of the utriculus and sacculus. Nevertheless, the epithelial part of the labyrinth is essentially like the teleostean labyrinth in general except in the form of the recessus utriculi and the structure of the macula acustica utriculi.

The labyrinth lies in the auditory recess suspended there by the thickenings and processes of the compact perilabyrinthine tissue. The utriculus occupies the lateral and anterior part of the recess. The recessus utriculi is enlarged transversely into a flattened somewhat wedge-shaped chamber. The narrow part of the chamber extends a little under the brain mesially while the broader part fits up under the arch made by the falciform process and the projecting part of the pterotic bone, and is thus in relation by its lateral surface to the lateral recess of the skull; ventrally, the surface of the recessus utriculi rests directly on the lips of the slit-like fenestra of the anterior bony capsule (fig. 12). Sections show conclusively that no part of the labyrinth enters the fenestra. What Weber and Ridewood mistook for a utricular diverticulum is merely the tissue space in the upper chamber of the osseous capsule above the septum. Under the recessus utriculi is the subcerebral perilabyrinthine canal, which, as described above, is here subdivided into an anterior and posterior canal (*USCA*, *USCP*) by the projecting lips of the fenestra as a result of their attachment to the floor of the utriculus.

The macula acustica utriculi is differentiated into three parts which run transversely across the floor of the broad wedge-shaped recessus utriculi; the anterior division (fig. 17, *MAA*) lies along the line of attachment of the floor of the recessus utriculi to the anterior lip of the slit-like fenestra of the anterior osseous capsule; the middle division (*MAM*) runs along the line of attachment to the posterior fenestral lip; the posterior division (*MAP*) lies a little farther back, and in no particular relation to structures outside the utriculus. These three divisions slightly diverge laterally; mesially they unite in a common area which lies in the narrow part of the wedge-shaped recessus utriculi nearly over the mesial end of the fenestra. Under each division of the macula, the tunica propria is rather thick and contains the branches of the eighth nerve which supply the macular cells. The middle band of the thickened tunica propria under the middle division of the macula is shaped like a three-sided prism which is attached by its edge to the posterior

lip of the fenestra by very delicate connective-tissue strands which are usually torn off in dissection. This band of tunica propria and its overlying macular cells projects about equally over the opening of the fenestra and over the posterior division of the utricular subcerebral canal (fig. 17). The tunica propria of the anterior division of the macula is thinner and band-like and is loosely attached to the edge of the anterior fenestral lip.

The anterior and middle divisions of the macula consist of band-like areas of a two-layered epithelium (fig. 19). The basal layer is composed of small cells with small round, dense nuclei; the upper layer is of columnar cells, in which the cytoplasm distal to the nuclei is densely granular and with indistinct cell walls. Under the cuticulum of each cell is a small round, dense body staining heavily with iron hematoxylin. Running out from this body is a rather long cilium which is quite stout and stains deeply near its origin.

The anterior and middle divisions of the macula are separated by only a slight interval and they lie in planes which are nearly at a right angle to each other. Bridging across this angle and connecting the two macular surfaces through the endolymph, is a structure which in the early and adolescent stages of development appears as a thick hyaline plate. In the adult this plate has become transformed into an otolith by the deposit of calcareous granules within it or on its upper surface. Hence, I shall refer to the structure as the otolithic membrane of the macula acustica utriculi. Its shape is determined by the interval between the two macular divisions, and hence it forms a long narrow triangle. Its apex is rounded mesially where the two divisions of the macula unite; laterally it ends in a free edge. The other edges spread out to cover the two macular surfaces.

The middle part of this plate in the adolescent stages of *Stolephorus* appears to be hyaline and structureless with the exception of some horizontal striations. On each side of the middle, the membrane appears to contain a great number of cells or chambers like a honeycomb which end blindly toward the middle of the membrane, but open onto the macular surfaces. The plate undergoes much shrinkage during histological prepa-

ration, and hence is always seen pulled off from one or both of its surfaces of attachment. There are often, however, attaching strands which extend down to the surface of the macular cells (particularly in the case of the anterior division).

The relation of the otolithic membrane to the labyrinth epithelium and to the endolymph at once suggests a comparison with the tectorial membrane of the mammalian ear. According to the conclusions of Prentiss ('13), the latter is primitively a cuticular organ of a chambered structure which is secreted between and at the ends of the cells composing the basal epithelium of the cochlea. The otolithic membrane in Clupeoids is probably formed by an analogous process.

This chambered structure appears in sections of the organ in all specimens from about 6 mm. up. In figure 20 is shown a section through that part of the otolithic membrane which covers the middle division of the macula; the section passes nearly parallel to the surface of the latter, and hence at right angles to the chambers. The section appears as a plate perforated with round holes. Near its margins the holes become oval and then elongated and less distinctly visible, due doubtless to the more oblique angle at which the chambers are sectioned. When studied by means of the high-power binocular microscope with oblique substage illumination, a dark rim always appears on one side of each hole. By rotating the decentered substage diaphragm the dark rim is seen to move evenly around the margin of each hole. The only probable interpretation seems to be that the dark rim is a refractive phenomenon and the holes are circular, each with a smooth even circumference. I was unable to demonstrate sections of cilia in these holes. If account is taken of the amount of shrinkage which the membrane undergoes in preparation, it is fair to assume that these chambers have been considerably enlarged by the shrinkage process. This consideration and the circular shape of the sections of the chambers suggest that they may in life be just large enough to accommodate the cilia. Probably, then, the substance of the membrane begins as a cuticulum, and is further developed by secretion from the tops of the macular cells, and in this secretion the

cilia are embedded. The chambers as they appear in fixed material are produced by shrinkage of the membrane substance away from the cilia.

De Beaufort has briefly mentioned the macula acustica in the Clupeidae. He says ('09, p. 619): "Mit dem utriculus existiert daher keine Verbindung, wohl liegt aber seine ventrale Fläche mit der Macula acustica oberhalb der erwähnten Öffnung der Bulla." Tysowski gives a figure ('09, fig. 1) in which the macula is shown lying over the fenestra of the bulla. His figure seems to show the division of the organ into three parts, but he makes no mention of it in his text.

THE FUNCTION OF THE EAR-SWIMBLADDER RELATION IN CLUPEOIDS

The swimbladder has a complexity of function correlated with the diversity of its anatomical structure. Bridge and Haddon ('93) thus summarize the functions which have been attributed to the organ in different species of fishes: 1) phonation, 2) respiration, 3) accessory to audition, 4) purely hydrostatic. The swimbladder of many species probably has more than one of these functions, but different functions in varying degrees. Hence, its physiology is a complicated problem which requires for complete solution a separate investigation in each of the structural types of the organ. Results of experimental work on fishes with a certain type of the organ can hardly be assumed to apply in every case without modification to fishes having a different type. Moreau ('76) and Baglioni ('08) have laid the foundation of our knowledge of the general physiology, but on the function of the specialized types of the swimbladder very little recorded experimental work can be found. An exception to this statement is the work of Tower ('08) on the sound-producing function of the organ in certain groups of fishes.

The ear in fishes is also an organ of complicated physiology. Audition and equilibration are functions usually assigned to this organ, but experimental evidence on the differentiation of functions in the different parts of the membranous labyrinth is incomplete (Lee, '98; Parker, '08).

The physiological problem is even more complicated when it is a question of the function of these two organs, brought into relation to each other by a definite mechanism. Is one organ accessory to the other, and, if so, which of the several functions of one organ does the other subserve? Or, is some entirely new function evolved as the result of their interrelation by the development of a connecting mechanism?

Most of the anatomical papers on the ear-swimbladder relation offer suggestions and sometimes extended discussions on the physiology of the mechanism, but it seems remarkable that in a century since Weber no experimental work on this unique relation has been recorded. The theories suggested cannot be discussed here in detail, but the most important of them may be summarized as follows: 1) accessory to hearing (Weber); 2) perception of hydrostatic pressures (Hasse); 3) appreciation of changes in barometric pressures (Sagemahl). Bridge and Haddon ('93), in an important paper on the Weberian ossicles, have shown that the anatomical structure of this mechanism is not consistent with the first or third theory, but is adapted to perception of changes in hydrostatic pressures. Wright ('84) came to the same conclusion.

With regard to the physiology of the Clupeoid type of the ear-swimbladder relation, little has been written. At the present time, however, the anatomical relations of these structures are perhaps sufficiently well known to justify the statement of a physiological theory, which it is to be hoped may be confirmed or modified later by experimental work.²

² The lateral recess of the skull would appear to furnish an easy means of reaching the ear-swimbladder mechanism in Clupeoids in experimental work (fig. 12). This recess is separated from the outside only by the lateral wing of the frontal bone which is quite superficial. The Clupeoids, however, or at least the American species, seem in general unfavorable for physiological experimentation. They are free-swimming in their habit and hence do not thrive when confined in small enclosures. They are delicate and of a 'nervous temperament,' so to speak, and they do not easily adjust themselves to handling and to operative procedures. Fishes with the Weberian ossicles, e.g., siluroids and certain cyprinoids, would appear to be much more favorable for experimental study of the ear-swimbladder relation.

The mechanical relation of the swimbladder to other structures is shown diagrammatically in figure 8. The swimbladder is a tube of very small diameter in comparison with the size of the body of the fish and with the thickness of its muscular wall; it has no direct connection with the skin or other superficial structures, as is the case in many other groups of fishes (with the exception in some species of a postanal connection with the exterior, which will be discussed below). It may therefore be assumed that the thick muscular wall of the body, supported by vertebrae, ribs, scales, spines, etc., interferes with the direct and immediate transmission of changes in hydrostatic pressure from the surrounding water to the swimbladder. In the head, however, the anatomical structure indicates that there exists a pathway for the transmission directly to the anterior membranous vesicle of changes in the outside pressure resulting from movement of the fish from one water level to another.

The pressure transmission from the outside to the walls of the vesicle may be assumed to take the following pathway (indicated by the arrows *A*, *A1*, *A2*, fig. 8): from the lateral-line canal in the lateral recess to the lateral perilabyrinthine spaces, thence to the whole system of perilabyrinthine canals, particularly to the utricular perilabyrinthine canal (with which the lateral spaces are in direct connection), thence through the two parts of that canal, around the lips of the fenestra, to the floor of the recessus utriculi, then through the endolymph down through the fenestra— and through the superior chamber of the osseous capsule and the elastic septum to the wall of the anterior membranous vesicle. Since the walls of the gas-filled vesicle beneath the septum are thin and loosely attached to the sides of the inferior chamber of the bony capsule, the vesicle easily expands and contracts, and hence pressure transmission from the outside to it will cause a mass movement of fluid along the pathway indicated. The parts of the utricular wall immediately adjacent to and in between the anterior and posterior divisions of the macula are thin with little or no condensed tissue. The thin floor of the utriculus between the two divisions of the macula overlies the fenestra, and hence is in the

direct pathway of the transmission of pressure. Since each of the macular divisions is very loosely attached to the edge of the fenestral lips, the fluid movement resulting from the transmission of force through the fenestra may cause a slight motion of the divisions of the macula which will result in a stimulation of the macular cells by the cilia embedded in the otolithic membrane.

A possible objection to this theory of the mechanics of these structures as stated above may be alleged, owing to the fact that in some of the Clupeoid species the caudal end of the swimbladder opens directly to the exterior just behind the anal opening. In the majority of the Clupeoids and allied forms, however, the postanal connection of the swimbladder with the exterior is not present. De Beaufort ('09) names nine species with an opening near the anus and fifteen without such an opening. Of the American species (not included in the list of de Beaufort), *Alosa sapidissima*, *Brevoortia tyrannis*, and *Stolephorus mitchilli* have no anal communication. In species in which the opening is present the communication takes place through a slender thin-walled tube which curves around to the left between the genital and kidney ducts to the left body wall and opens to the exterior between the genital opening and the anus (de Beaufort, '09, described in *Clupea harengus*). It might be said that the expansion or compression of the gas in the swimbladder may take place directly through the postanal opening (in those species in which it exists) and thereby balances the pressure transmitted to the wall of the anterior membranous vesicle along the pathway indicated above to such a degree that pressure transmission through the head would not be effective in originating a stimulus in the divisions of the macula. Possibly this may be true to some degree for extreme and very sudden changes in the hydrostatic pressure of the surrounding medium. It seems hardly probable, however, that the transmission of changes in hydrostatic pressure through a slender, curved, thin-walled tube could be as direct or as immediate as through the perilabyrinthine spaces. The possibility of the transmission of changes in hydrostatic pressure to the

body of the swimbladder either directly through the anal region or even indirectly through the fluid-permeated tissues of the body of the fish cannot be denied. But the requirements of the theory as stated above are sufficient if it can be shown that transmission of the pressure changes through the lateral recess and the perilabyrinthine canals to the anterior membranous vesicle is more direct, more immediate, and more sensitive to slight variations than the transmission of pressure changes through the body walls or through the postanal opening to the body of the swimbladder.

An auditory function may possibly be attributed to this mechanism. This was the view expressed by most of the older anatomical writers. Weber (1820), for example, considered that the septum in the anterior osseous capsule functions as a tympanic membrane. The anatomical structure of this mechanism hardly appears to support this view. To be effective as an aid to hearing, it must be constructed so as to transmit molecular vibrations from the outside to the membranous labyrinth more quickly or more efficiently than they can be transmitted by other existing structures in the head of the fish. Whereas, it would appear that the molecular vibrations of sound waves would pass more easily to the membranous labyrinth directly from the water through the bones and fluid-permeated tissues of the head. This apparatus is so constructed that we have a pathway adapted for a mass motion of fluid; it can hardly be considered as a means of increasing efficiency of transmission of the molecular motion of sound waves. These considerations are similar to those which lead Bridge and Haddon to conclude that the Weberian ossicles are for the transmission of hydrostatic pressures and not accessory to audition.

If the functional significance of these structures can be shown to be essentially as stated above, the anterior and posterior divisions of the macula acustica utriculi are together to be considered as a 'depth sense organ' or, more accurately speaking, a receptor which is stimulated by changes in hydrostatic pressure which usually, or perhaps always in the normal life of the fish, result from movements from one water level to another.

The anterior membranous vesicle enclosed in the osseous capsule, the enlargement of the lateral-line canal in the lateral recesses, the lateral recess of the skull, the perilabyrinthine canals, and the otolithic membrane are to be considered as accessory structures by which the stimulus is brought to bear on the receptor.

This mechanism as a whole may be conceived to function in one of the following ways:

1. Merely as a sense organ which acquaints the fish with changes in water level.
2. As a part of a reflex mechanism actuated by changes in water level, originating nerve impulses which pass out by efferent fibers of the spinal nerves to the skeletal muscles, thereby producing compensatory movements which tend to maintain the fish at a nearly constant water level.
3. As a part of a reflex mechanism which through efferent visceral nerves transmits nerve impulses to the secretory cells and smooth muscle fibers in the swimbladder wall and which thus adjusts the swimbladder to changes in hydrostatic pressure.
4. By some combination of two or possibly of all three of these functions.

To consider this ear-swimbladder mechanism "merely as a sense organ" (whatever that term may mean) is hardly adequate in view of our present conceptions of the nervous system in general and the nerve connections of the membranous labyrinth in particular. In all vertebrates the maculae and cristae seem to function primarily (and possibly exclusively) through reflex mechanisms. It is probably safe to affirm this positively in case of the lower vertebrates. It is therefore almost certain that this apparatus acts reflexly according to the second or third possibility as stated above or by combination of the two. We know, through the work of Moreau, Baglioni, and others, that in the case of the Physoclisti the gas pressure inside the swimbladder is adjusted to changes in the outside water pressure as a result of an interaction between the absorptive and secretory mechanisms in the wall of the organ. In the Clupeoids there is an epithelium, apparently secretory, in the pneumatic duct and probably in the tubular portion of the

precoelomic diverticulum; smooth muscle surrounds the opening of the diverticulum to the body of the swimbladder and is also found in the walls of the pneumatic duct which opens into the stomach. Apparently, therefore, a mechanism for the adjustment of the gas pressure in the swimbladder exists; the nerve impulses controlling it are doubtless of a reflex nature and, in the absence of experimental evidence to the contrary, it seems a reasonable hypothesis that the source of the afferent impulses of this reflex is in the divisions of the macula recessus utriculi.

That this mechanism is effective in maintaining the fish at a nearly constant level is scarcely consistent with what little is known regarding the habits of these fishes.

It is known that herring and menhaden, within a few minutes, will dive below the surface to depths greater than the bottom of the purse seines of the fishermen. Possibly, however, while this mechanism may not suffice to force the fish to remain near one level, it may by compensatory motions of fins and body musculature tend to prevent the fish from departing far from a certain level except in extreme cases.

This apparatus, however, may be effective in maintaining the fish at certain water levels within certain wide limits. The mechanism of secretion and absorption of gas in the body of the swimbladder probably has its physiological limits; it is possible that the mechanism described may prevent the fish from descending to depths greater than those at which the fish is able to adjust the gas pressure in the body of the swimbladder to the hydrostatic pressure.

Anatomical work, however, can hardly go further than the statement of the problem and the discussion of its possibilities. Further analysis of the function of this mechanism can only be attained by experimental work.

SUMMARY

1. The swimbladder in Clupeoids is composed of a mucosa (tunica interna), a submucosa, and an outer dense connective-tissue layer (tunica externa).

a. In the body of the swimbladder and in the membranous vesicles, the lining is simple squamous epithelium, but in the pneumatic duct and in the precoelomic diverticulum the epithelium is columnar and apparently glandular in structure.

b. A circular layer of smooth muscle is found around the pneumatic duct and at the beginning of the precoelomic diverticulum.

c. The glandular cells and the smooth muscle probably regulate the gas pressure in the swimbladder.

2. The perimeningeal tissue is differentiated in certain places into a compact elastic tissue (perilabyrinthine) which extends away from the walls of the labyrinth under the brain (subcerebral plate) and in a band over the medulla just behind the cerebellum (supracerebral band). Less extensive developments of this tissue are found in relation to other parts of the labyrinth.

3. A complicated system of intercommunicating canals and spaces of rarefied connective tissue extends through the perilabyrinthine tissue around the brain and labyrinth. These spaces may be grouped in three divisions:

a. Lateral, spaces in the tissue in the lateral recess near the lateral-line canal and lateral to the labyrinth.

b. Subcerebral, two canals traversing the under surface of the subcerebral perilabyrinthine plate; the posterior of these canals (saccular subcerebral canal) connects the saccular portions of the auditory recesses of the two sides; the anterior canal (utricular subcerebral canal) connects the lateral recesses of the two sides of the skull by passing under the utriculus where it is partially subdivided into two canals by the projection of the lips of the fenestra of the anterior osseous capsule.

c. Supracerebral, the canal in the supracerebral perilabyrinthine band over the medulla.

4. The floor of the recessus utriculi rests on the lips of the fenestra and is attached to them by delicate connective-tissue strands. The utriculus does not send a diverticulum into the superior chamber of the anterior osseous chamber.

5. The upper chamber of the anterior osseous capsule contains very sparse connective tissue and is similar in structure to the perilabyrinthine spaces.

6. The macula recessus utriculi on the floor of the recessus utriculus is subdivided into three parts (anterior, middle, and posterior). The anterior and middle parts of the macula are so situated that they lie along the line of attachment of the floor of the recessus utriculi to the anterior and posterior fenestral lips, respectively.

7. The space between the anterior and middle divisions of the macula is bridged by the otolithic membrane in which the cilia of the macular cells are probably embedded.

8. The otolithic membrane originates from the cuticulum over the macular cells. Probably its further development takes place by the secretion of substance from the surface of the macular cells.

9. The perilabyrinthine canals form a system of spaces through which changes in hydrostatic pressure of the water are transmitted to the thin walls of the anterior membranous vesicle. The mechanical relations are such that this transmission of pressure causes a slight motion of the anterior and middle divisions of the macula acustica utriculi, and a consequent stimulation of the cells by means of the cilia embedded in the otolithic membrane.

10. The anterior and middle divisions of the macula acustica utriculi constitute a receptor stimulated by changes in hydrostatic pressure resulting in the movement of the fish from one water level to another.

11. Impulses arising from this receptor probably originate the reflex which controls the gas-regulating mechanism in the swimbladder. They may also give rise to reflexes which produce compensatory movements of the swimming musculature and thereby maintain the fish within certain water levels.

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FIGURES

ABBREVIATIONS

- A, A¹, A²*, arrows in figure 8 to show the probable direction of transmission of hydrostatic pressure from the outside to anterior membranous vesicle
- AO*, aorta
- AOC*, anterior osseous capsule
- ASCA*, ampulla of anterior semicircular canal
- ASCL*, ampulla of lateral semicircular canal
- ASP*, alisphenoid bone
- AV*, anterior membranous vesicle
- BAOC*, bone of anterior osseous capsule
- BR*, brain
- BSO*, basioccipital bone
- CA*, crista acustica in ampulla of the anterior semicircular canal
- CC*, cartilage of the canal of the swimbladder diverticulum
- CI*, inferior chamber of anterior osseous capsule
- COC*, cartilaginous otic capsule
- CS*, superior chamber of anterior osseous capsule
- CSD*, canal of the swimbladder diverticulum in the cranium
- E*, eye
- EMV*, Epithelium of the membranous vesicle of the swimbladder
- EPO*, epiotic bone
- EXO*, exoccipital bone
- F*, fenestra
- FB*, fusiform bulla of the pre-coelomic diverticulum of the swimbladder
- LC*, lateral-line canal
- LF*, lip of the swimbladder of the fenestra
- LR*, lateral recess of the skull
- MAA*, anterior division of macula acustica utriculi
- MAM*, middle division of the macula acustica utriculi
- MAP*, posterior division of the macula acustica utriculi
- MD*, medulla
- MV*, embryonic membranous vesicle of precoelomic diverticulum
- NAM*, nerve of the anterior division of the macula acustica utriculi
- NPM*, nerve to the posterior division of the macula acustica utriculi
- OM*, otolithic membrane
- P*, periosteal lining of the anterior osseous capsule continued on the surface of the septum
- POC*, posterior osseous capsule
- PRO*, prootic bone
- PSB*, precoelomic diverticulum of the swimbladder
- PSC*, supracerebral perilabyrinthine canal
- PSCL*, branch of supracerebral perilabyrinthine canal which connects with the perilabyrinthine spaces of the lateral recess
- PSCM*, branch of the supracerebral perilabyrinthine canal which opens medially to the sacculus
- PV*, posterior membranous vesicle
- RU*, recessus utriculi

- RX*, recurrent ramus of vagus nerve
S, sacculus
SB, body of the swimbladder
SC, septum of anterior osseous capsule
SCA, anterior semicircular canal
SCL, lateral semicircular canal
SCP, posterior semicircular canal
SPA, sphenotic bone
SPP, subcerebral plate of perilyrinthine tissue
SS, superior sinus of the utriculus
SSC, saccular subcerebral perilyrinthine canal
TPEXO, triangular plate of the exoccipital bone
TPP, triangular plate of perilyrinthine tissue lateral to utriculus and sacculus ('accessory bulb' of Breschet)
USC, utricular subcerebral perilyrinthine canal
USCA, utricular subcerebral perilyrinthine canal anterior to the lips of the fenestra
USCP, utricular subcerebral canal posterior to the lips of the fenestra
VN, vein
VT, vertebral column
VI, abducens nerve
VIII, acoustic nerve
IX, glossopharyngeal nerve
 *, ridge in thickened roof of utriculus which is continuous with the perimeningeal tissue lining the lateral wall of the cranium

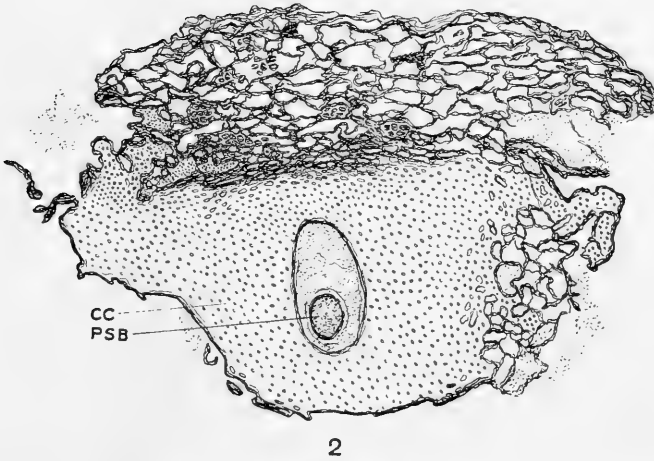
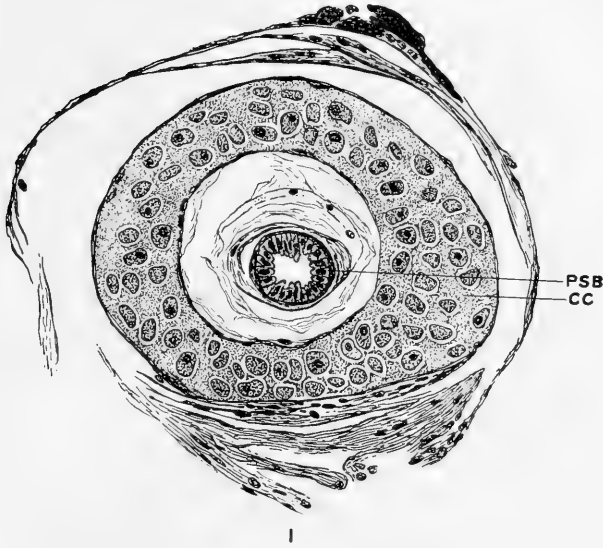


Fig. 1 Section through the cartilage canal and the precoelomic diverticulum of the swimbladder of *Stolephorus mitchilli*.

Fig. 2 Section through the cartilage canal and the precoelomic diverticulum of the swimbladder of *Alosa sapidissima*.

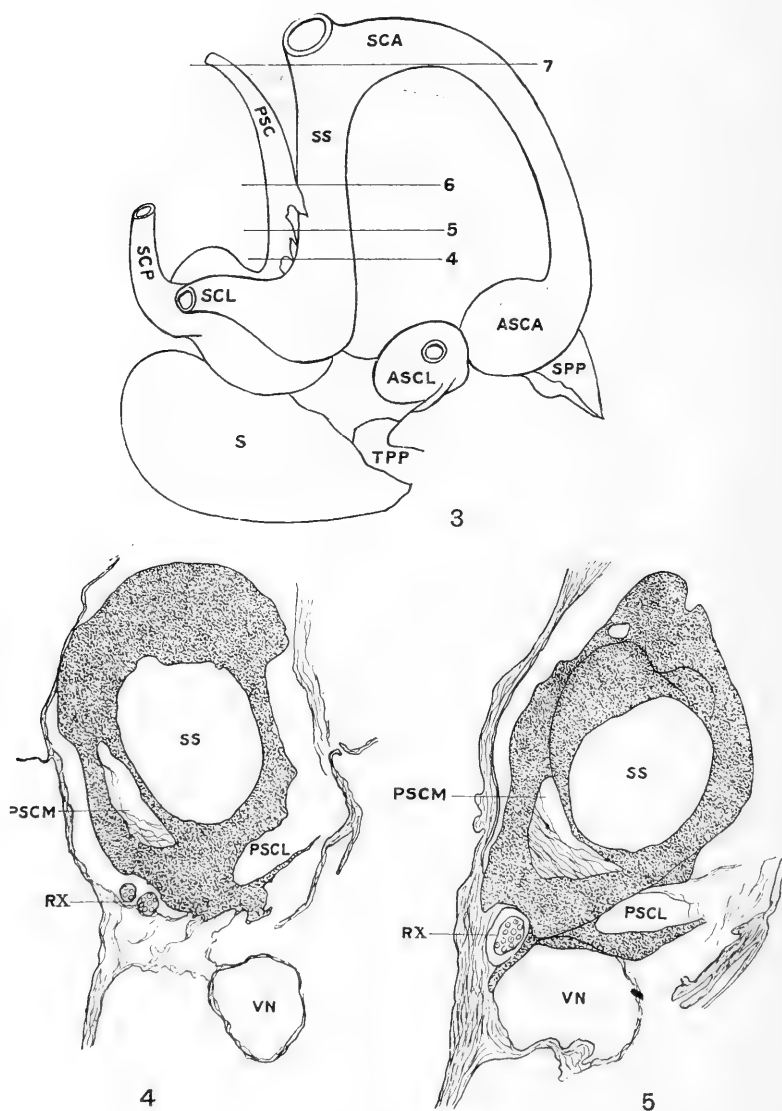


Fig. 3 Diagram of the right membranous labyrinth and supracerebral perilyabyrinthine canal of *Alosa sapidissima*, based on figure 12; orientation diagram of the approximate planes of the sections shown in figures 4, 5, 6, and 7.

Figs. 4, 5, 6, and 7 Sections through the right labyrinth and supracerebral perilyabyrinthine canal at levels shown in the orientation figure (fig. 3).

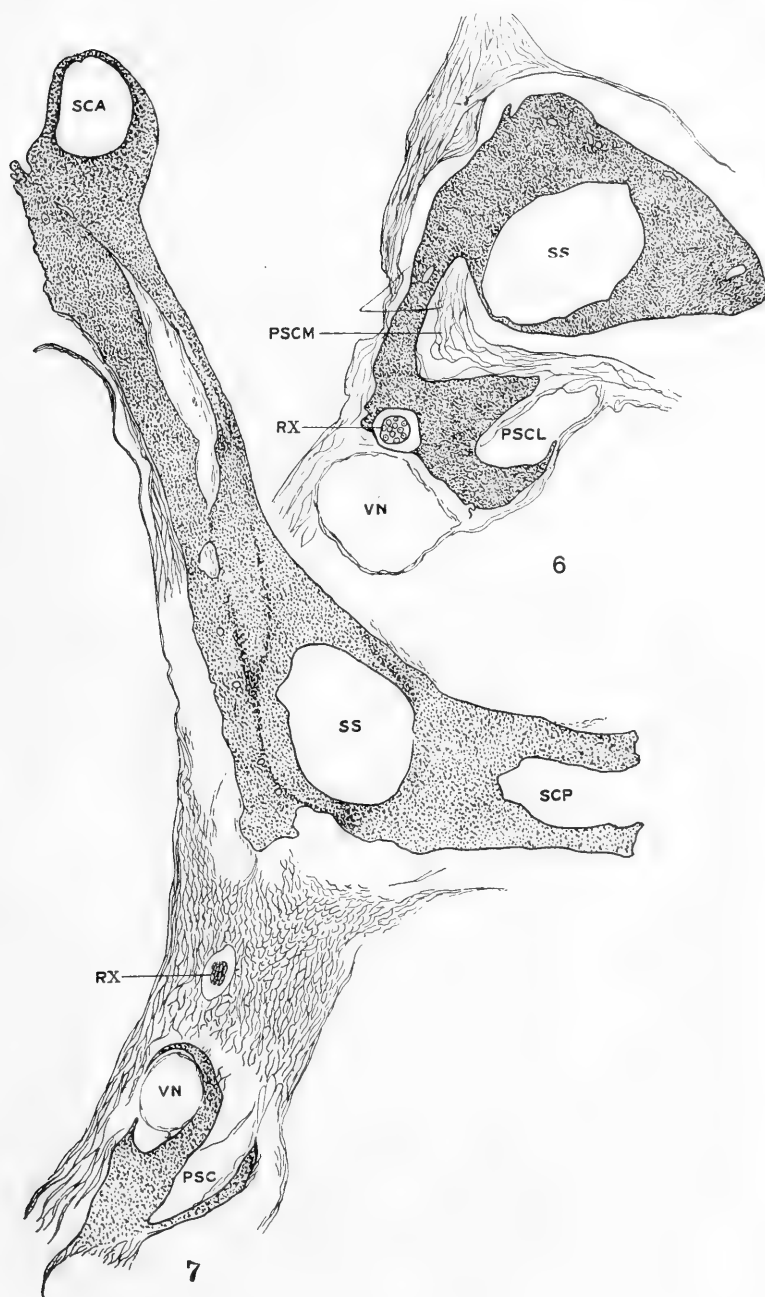


Fig. 8 Diagrammatic drawing to show the relation of the swimbladder to the body and head in the Clupeoids. The arrows, A, A1, and A2, show the direction of transmission of changes in pressure from the outside to the anterior membranous vesicle.

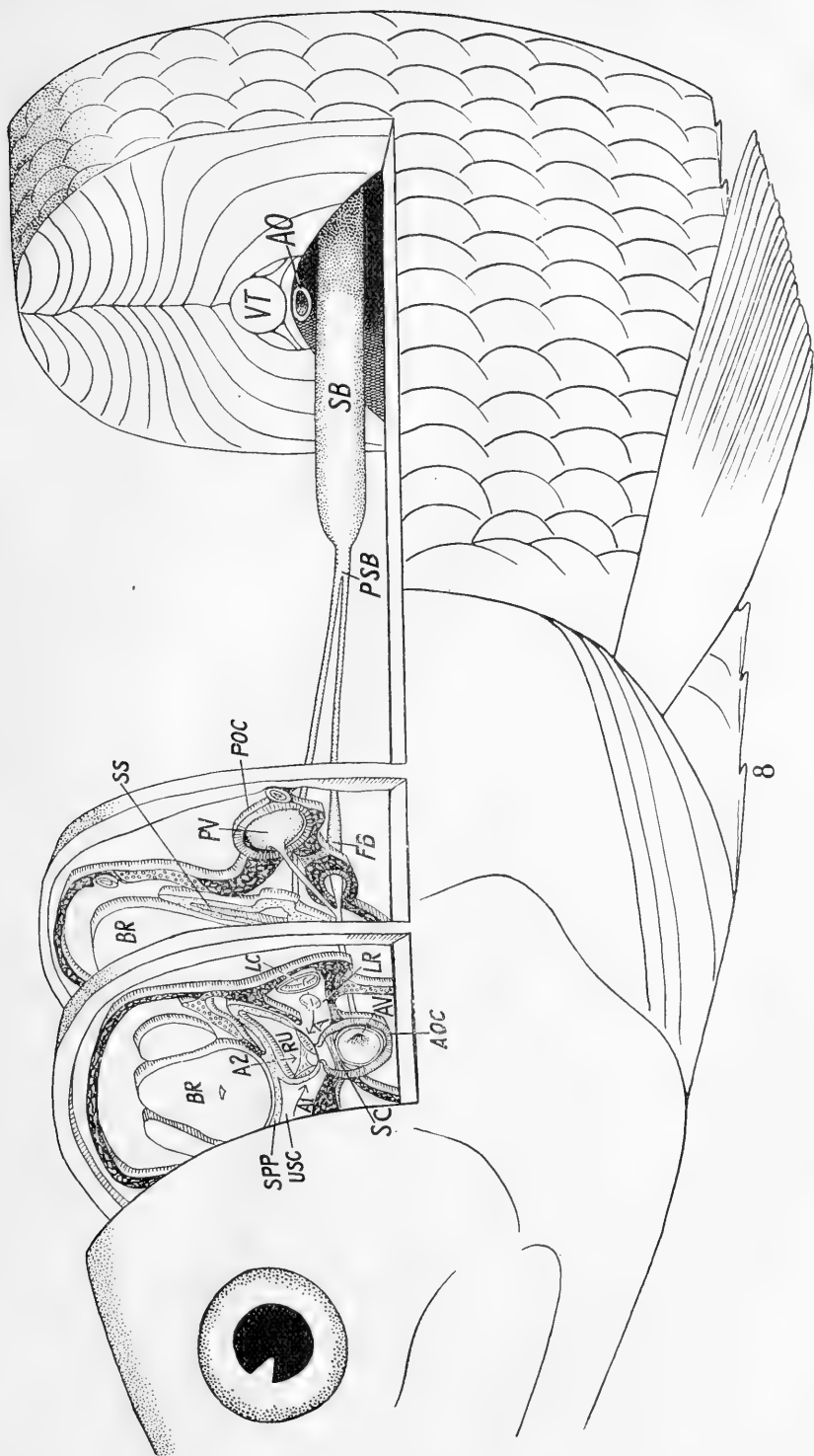


Fig. 9 Dissection from the right ventrolateral side of the skull to show the relation of the membranous labyrinth to the anterior osseous capsule, also the relation of the anterior and middle divisions of the macula acustica utriculi to the lips of the fenestra. *Pomolobus pseudoharengus*.

Fig. 10 Drawing of the ventrolateral side of a reconstruction model of the left labyrinth and of the single embryonic membranous vesicle. *Stolephorus mitchilli*, 28 mm. in length. The three cristae acusticae in the ampullae of the semicircular canals are shown. The membranous vesicle is drawn transparent, and through it can be seen the three divisions of the macula acustica utriculi. The sacculus projects downward and backward.

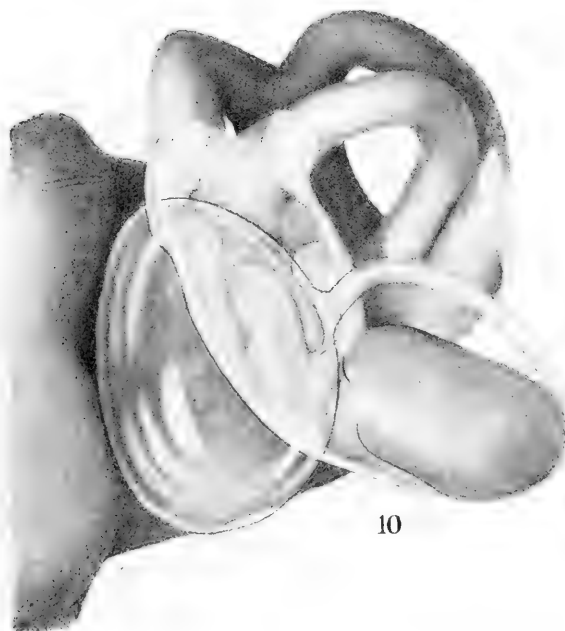
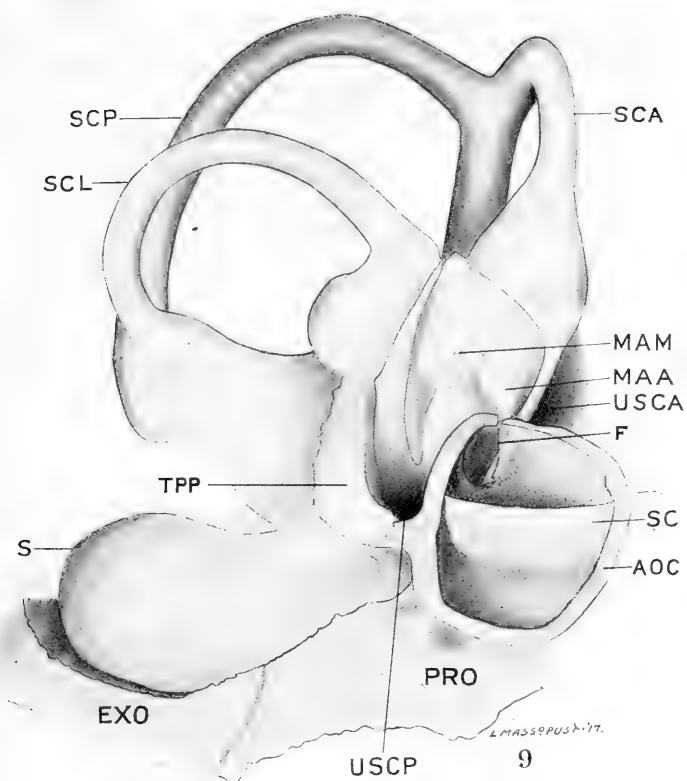


Fig. 11 Drawing to show the membranous labyrinth and the subcerebral perilabyrinthine plate and canals in relation to the floor and side of the skull. Drawing somewhat diagrammatic, based on dissections and a model. The upper half of the cranial wall and of the semicircular canals have been dissected away. The specimen is cut in the median sagittal plane which shows at the right side of the figure; a second cut parallel to this and about half-way to the outside separates the specimen into two parts which are represented as being slightly separated. In the median plane are shown the utricular and saccular subcerebral canals; in the second plane are shown the relations of the utricular subcerebral canal where it is divided into two parts by the lips of the fenestra of the anterior osseous capsule; the two parts of the canal connect laterally under the utriculus with the lateral recess of the skull (fig. 12). The relation of the recessus utriculi to the fenestral lips and to the two chambers of the anterior osseous capsule are also shown. The canal for the precoelomic diverticulum is cut obliquely just before its entrance to the capsule. The cavity of the posterior osseous capsule is cut into slightly on its median side. *Pomolobus pseudoharengus*.

Fig. 12 Lateral dissection of the labyrinth and perilabyrinthine structures. The utriculus rests on the lips of the fenestra with a division of the utricular subcerebral canal (*USCP*, *USCA*) on either side. These relations are those seen on looking medially toward the utriculus when the lateral recess of the skull is opened up from the outside. Posteriorly in the drawing, the supracerebral perilabyrinthine band springs from the base of the superior sinus. Accessory plates of perilabyrinthine tissue can be seen applied to the outside of the utriculus, the ampullae, the superior sinus, and the upper end of the anterior semicircular canal. The triangular projection of perilabyrinthine tissue (*TPP*) is probably the 'bulbe accessoire' of Breschet. An opening through this (not shown in the figure) puts the lateral recess in communication with tissue spaces lateral to the superior sinus and hence with the supracerebral canal (*PSC*) through its irregular opening.

Fig. 13 Diagram of a sagittal section of the anterior osseous capsule and septum: orientation figure for figure 14. *Pomolobus pseudoharengus*.

Fig. 14 Drawing to show the histological detail of the septum and its attachment to the wall of the anterior osseous capsule. The elastic plates of which the septum is made up anastomose freely as they approach the bone and are attached by white connective-tissue fibers which pass into irregular radial canals in the bone. The periosteum of the two chambers is reflected onto the surfaces of the septum.

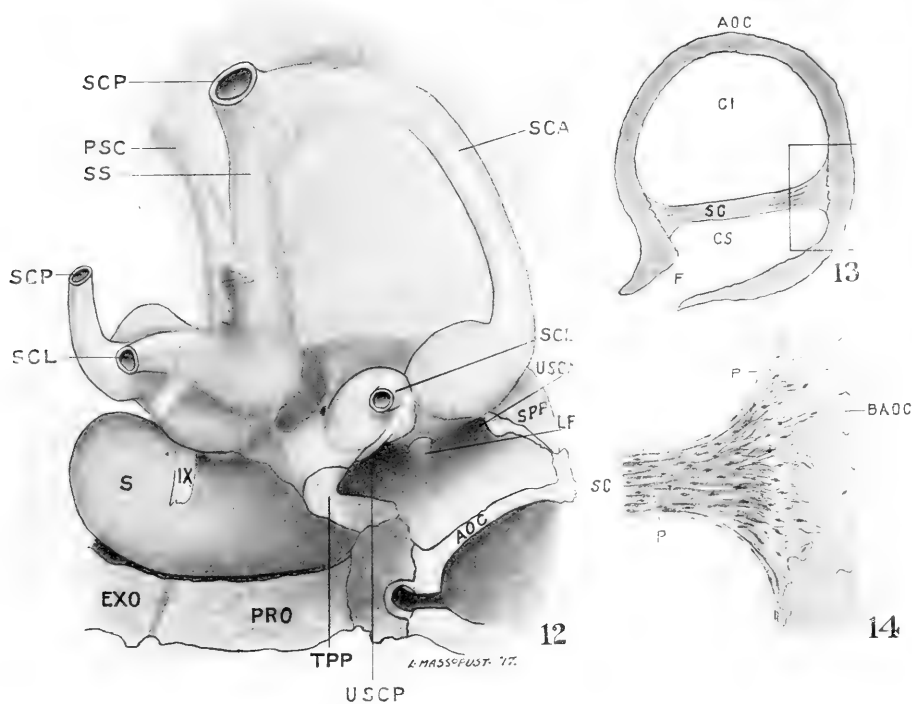
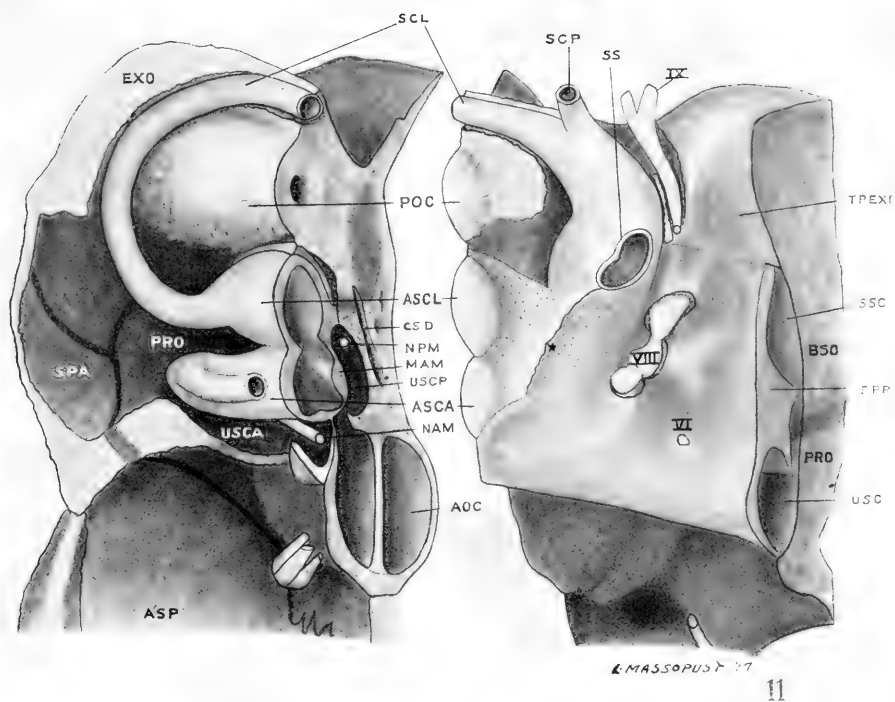


Fig. 15 Microscopic section of the head of *Stolephorus*, 28 mm. in length. The section is sagittal and is taken about one-third the distance between the median plane and the lateral side of the head. The otic capsule is still cartilaginous, though membrane bone formation has begun on the surface. There is only one membranous vesicle (*MV*) at this stage. The canal (*CSD*) for the pre-coelomic diverticulum passes through the cartilage of the otic capsule. The diverticulum itself is cut obliquely (*PSB*) just before its entrance to the membranous vesicle. The vesicle occupies a space excavated from the cartilage of the otic capsule; beginning of bone formation in the connective tissue around the vesicle has taken place.

Fig. 16 Dissection of the occipital region of the skull to show the position of the supracerebral perilabyrinthine band. *Pomolobus pseudoharengus*.

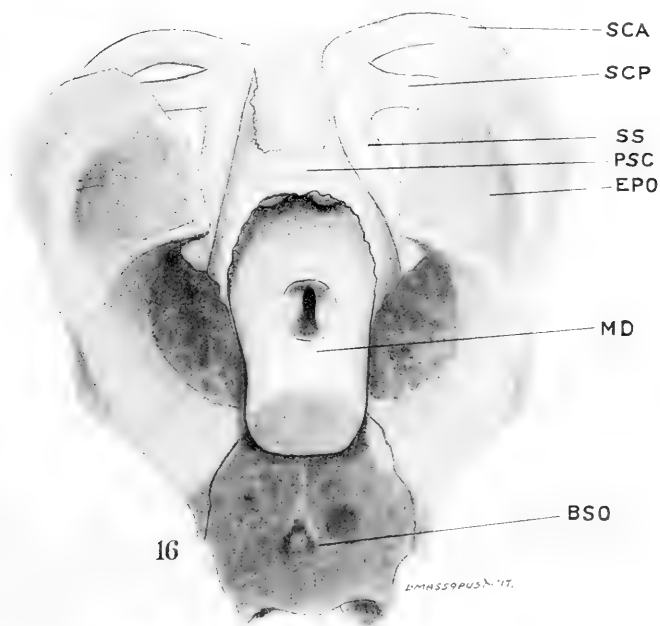
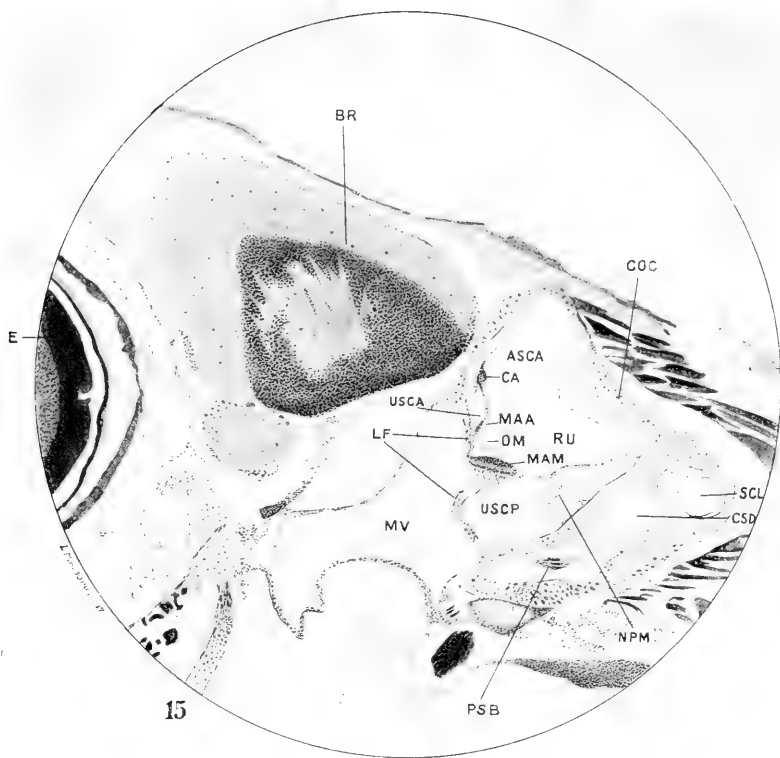
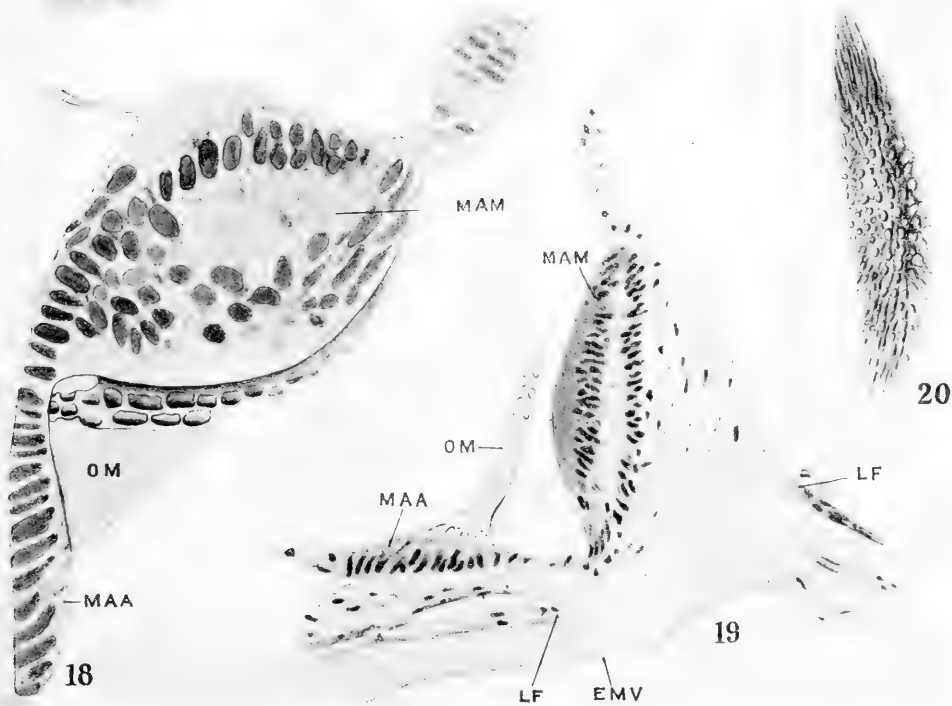
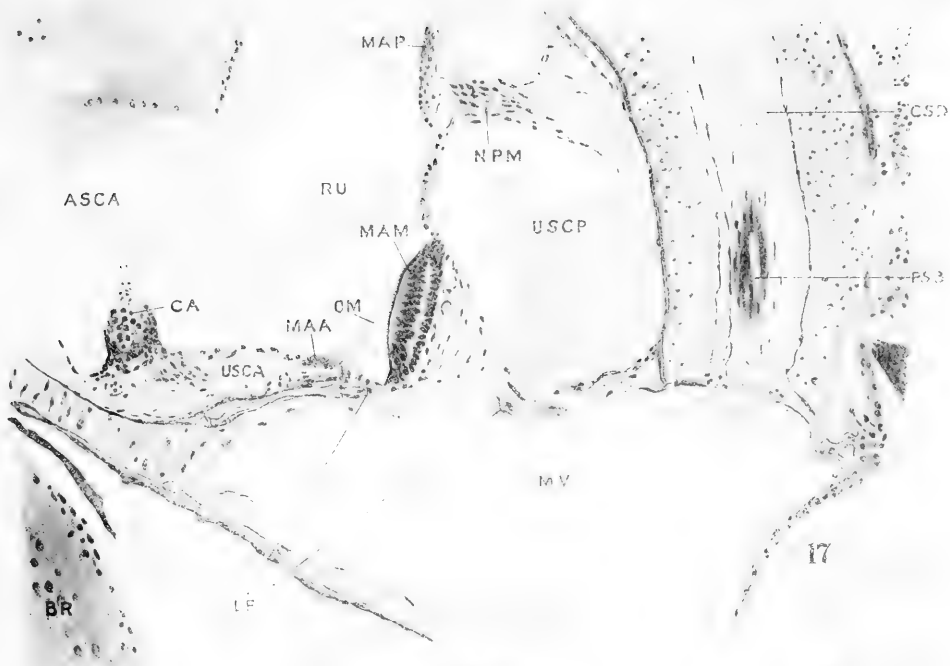


Fig. 17 A drawing from the same section shown in figure 15, but with higher magnification to show the relations of the three divisions of the macula acustica utriculi. The plane of section passes at the extreme lateral end of the posterior division of the macula. The septum of the anterior osseous capsule is only slightly developed at this stage and is not visible in this section. The relation of the anterior and middle divisions of the macula to the two divisions of the utricular subcerebral canal are well shown.

Fig. 18 Section of the anterior and middle divisions of the macula to show the structure of the otolithic membrane. *Stolephorus mitchilli*, 8 mm. in length. At this stage the divisions of the macula are in the process of differentiation from a single sensory spot. Section drawn as viewed through the binocular microscope, 1/12 oil-immersion lens. The otolithic membrane is slightly diagrammatic.

Fig. 19 Drawing from the same slide as figures 15 and 17 to show the details of the anterior and middle division of the macula acustica utriculi and of the otolithic membrane; 1/12 oil-immersion lens.

Fig. 20 Section of the otolithic membrane approximately parallel to its surface. *Brevoortia tyrannis*, 25 mm. in length, 1/6 objective. This shows the sections of the 'cells' or chambers in the membrane. They are cut transversely in the middle of the section, but appear oblique at either end owing to their change in direction.



Resumen por el autor, O. Larsell.
Universidades de Chicago y Wisconsin.

El cerebelo de Amblystoma.

El autor describe el cerebelo de Amblystoma con especial mención de sus progresos sobre la estructura y organización del cerebelo de los Urodelos inferiores. En Amblystoma existe una estructura mucho mas diferenciada, que corresponde a la posición mas elevada de este género en la serie de los anfibios. El cerebelo aumenta de tamaño y también en la complejidad de su estructura. Existe una capa de células de Purkinje, y lo mismo sucede con las células de los granos y otros rasgos típicos del cerebelo de los vertebrados mas superiores. Las conexiones de los tractos fibrosos se conservan relativamente simples.

Translation by José F. Nonidez
Carnegie Institution of Washington

THE CEREBELLUM OF AMBLYSTOMA

O. LARSELL

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TWENTY-ONE FIGURES

The cerebellum of the Amphibia is recognized as being of very primitive type. This is particularly true of the organ in the urodeles, in certain forms of which it is said by some authors to be entirely absent. Because of this primitive structure, a comparative study of the organ in various representative types of Amphibia should prove instructive. Such a study was made by Herrick ('14), with particular reference to *Necturus maculosus*, although *Amphiuma* means and *Cryptobranchus alleghaniensis* were also included, and some observations were made on the cerebellum of *Amblystoma tigrinum*.

It was at the suggestion of Professor Herrick that the present study was begun. It is a pleasure for the writer to acknowledge his sense of indebtedness to Professor Herrick for his interest and his generosity in making available every facility for the prosecution of the work, including a wax model of the hind-brain of larval *Amblystoma*, a series of sketches on cardboard of the brain stem of adult *Amblystoma tigrinum*, which had been cut to correspond with the outlines of the serial sections from which the plots were made, and loans of prepared slides. Acknowledgment should also be made to Dr. Paul S. McKibben for permission to use an extensive series of microscopical preparations, including brains of *Amblystoma*, both adult and larval, prepared by the Golgi, Weigert, Cajal, and other methods and mounted as serial sections. These preparations were made by Doctor McKibben and are his property. The opportunity to use them has very greatly facilitated the work on the cerebellum. In addition to these, a number of series of sections have been prepared by the

writer by various methods especially for this study. The macroscopic form and relations of the cerebellum have been determined by dissections under the binocular microscope. Three species of *Amblystoma*, namely, *tigrinum*, *punctatum*, and *opacum*, were employed in the study. The greater part of the description is based on *Amblystoma tigrinum* (Green).

The external form of the cerebellum of *Necturus* has been described and figured by Herrick in the paper previously cited. Kingsbury ('95), Miller ('00), McKibben ('13), and others, in descriptions or figures of the entire brain of this form, have also figured and described the cerebellum in greater or less detail. Other urodeles have received some attention in this respect, but so far as the writer is aware, but little description has been given of the cerebellum of adult *Amblystoma*. Stieda ('75) gives a brief description of this organ and a figure of the entire brain, including the cerebellum, in connection with his work on the central nervous system of *Siredon* (*Amblystoma mexicanum*). Osborn ('88) also figures the entire brain, with the cerebellum, of the Mexican axolotl, in a study of the internal structure of the brains of various urodeles, and Herrick ('14 a) figures it in connection with a detailed study of the medulla oblongata of larval *Amblystoma tigrinum*.

As compared with the cerebellum of *Necturus*, which has been described in greatest detail, this organ in *Amblystoma* is much more massive. This massive structure continues across the mid-plane, as pointed out by Herrick ('14). It forms a well-defined ridge which projects dorsocaudally, from the anterior end of the medulla oblongata, and which lies posterior to the points of emergence from the brain of the IV nerve (figs. 1 and 2). Below this ridge the IV ventricle extends laterally on either side to form the recessus lateralis. This recess continues forward, forming a blind sac which extends rostrad into the auricular lobe. This sac (fig. 3, *d.a.*) Herrick has called the anterior diverticulum. As compared with *Necturus* and *Amphiuma* and with larval *Amblystoma*, it is relatively small in the adult of the latter species. It is surrounded on all sides except posteriorly by massive tissue; mesally by the corpus cerebelli and auricular lobe; dorsally and

laterally by the auricular lobe, so that it forms in reality a continuation of the IV ventricle into the auricular lobe. The recessus lateralis is also covered, in adult *Amblystoma*, by massive tissue on all sides except caudodorsally, where the membranous chorioid plexus forms its roof. This is in contrast to the condition present in larval *Amblystoma*, in which the covering of the lateral recess is almost entirely membranous.

Laterally the main mass of the cerebellum is continuous with the auricular lobe and the rhomboidal lip of the medulla oblongata (figs. 1, 2, and 3). As pointed out by Herrick ('14) for urodeles in general, the relation of the cerebellum to the rhomboidal lip is very similar to that found in early embryonic stages of mammals. In *Amblystoma* there is relatively more massive structure than in lower forms of urodeles. The auricular lobe is closely related to the corpus cerebelli rostrally and dorsally. Johnston ('06), in summarizing the vertebrate cerebellum, states: "In most vertebrates the lateral walls bulge outward and forward as the auricular lobes, the floccular lobes in man." This describes the relation of the auricular lobe to the rest of the cerebellum in *Amblystoma*, with the reservation that the bulging is quite limited.

Herrick has defined the corpus cerebelli in *Necturus* as the cerebellar tissue which forms the anterior wall of the lateral recess and corresponds to the chief mass of the cerebellum in higher Amphibia and Reptilia, and the eminentia ventralis cerebelli as a forward extension of the eminentia trigemini which is continuous, in front of the lateral recess, with the corpus cerebelli. In *Amblystoma* there is apparent a pronounced eminence which projects posteriorly and downward into the ventricle, in the region corresponding to the corpus cerebelli of *Necturus*. This eminence (which represents only a part of the body of the cerebellum as Herrick used this term) is located medially of the anterior diverticulum (fig. 3, *c.cb.*). It is continuous with the eminentia cerebellaris ventralis, which in *Amblystoma* extends laterally and upward in such a manner as to form the principal part of the anterior wall of the lateral recess. As compared with *Necturus*, this entire region of the brain of *Amblystoma* appears telescoped

together anteroposteriorly, with upward displacement of cerebellar structures.

As will be more fully indicated later, the eminence formed by the corpus cerebelli contains a group of cells which appear to represent the foreshadowing of the nucleus dentatus of higher vertebrates. These cells are located in the anterior and ventral portion of the eminence, just medial to the dorsoventral plane of the anterior diverticulum. Because of the massive structures surrounding it on all sides except posteriorly, the anterior end of the fossa rhomboidea is greatly reduced in size, particularly in the region of the corpus cerebelli. Lateral to the anterior diverticulum the cerebellar structure is less massive and is continuous with the rhomboidal lip (fig. 3).

ABBREVIATIONS

<i>ax.</i> , axone	<i>Pur.c.</i> , Purkinje cell
<i>br.conj.</i> , brachium conjunctivum	<i>r.l.</i> , recessus lateralis
<i>caud.</i> , caudad	<i>r.P.c.</i> , reduced Purkinje cells
<i>cb.</i> , cerebellum	<i>r.VII l.l.d.</i> , radix lateralis facialis dorsalis
<i>c.cb.</i> , corpus cerebelli	<i>r.VII l.l.m.</i> , radix lateralis facialis medialis
<i>ceph.</i> , cephalad	<i>r.VII l.l.m'</i> , radix lateralis facialis ventromedialis
<i>com.cb.</i> , commissura cerebelli (myelinated component)	<i>r.VII l.l.v.</i> , radix lateralis facialis ventralis
<i>com.cb.l.</i> , commissura cerebelli lateralis (unmyelinated component)	<i>r.VIII</i> , radix nervi acustici
<i>d.a.</i> , diverticulum anterior of recessus lateralis	<i>str.gr.</i> , stratum granulare
<i>f.l.m.</i> , fasciculus longitudinalis medialis	<i>str.mol.</i> , stratum moleculare
<i>gr.c.</i> , granule cell	<i>str.Pur.</i> , stratum of Purkinje cells
<i>lat.</i> , lateral	<i>t.f.</i> , terminal fibers
<i>l.aur.</i> , lobus auricularis	<i>tr.a.</i> , dorsal longitudinal tract of area acustica
<i>lm.</i> , lemniscus	<i>tr.b.</i> , ventral longitudinal tract of area acustica
<i>mes.</i> , mesencephalon	<i>tr.m.cb.</i> , tractus mammillocerebellaris
<i>mes.V</i> , radix mesencephalica trigemini	<i>tr.sp.cb.d.</i> , tractus spinocerebellaris dorsalis
<i>m.fi.</i> , moss fibers	<i>tr.sp.cb.v.</i> , tractus spinocerebellaris ventralis
<i>m.s.p.</i> , midsagittal plane	<i>tr.sp.t.</i> , tractus spinotectalis
<i>n.III</i> , nervus oculomotorius	<i>tr.v.a.</i> , tractus visceralis ascendens (secondary gustatory tract)
<i>n.IV</i> , nervus trochlearis	<i>v.m.a.</i> , velum medullare anterior
<i>n.V</i> , nervus trigeminus	
<i>n.VII + VIII</i> , nervi facialis et acusticus	
<i>n.IX</i> , nervus glossopharyngeus	
<i>nuc.dent.</i> , nucleus dentatus	
<i>nuc.mes.V</i> , nucleus mesencephalica trigemini	

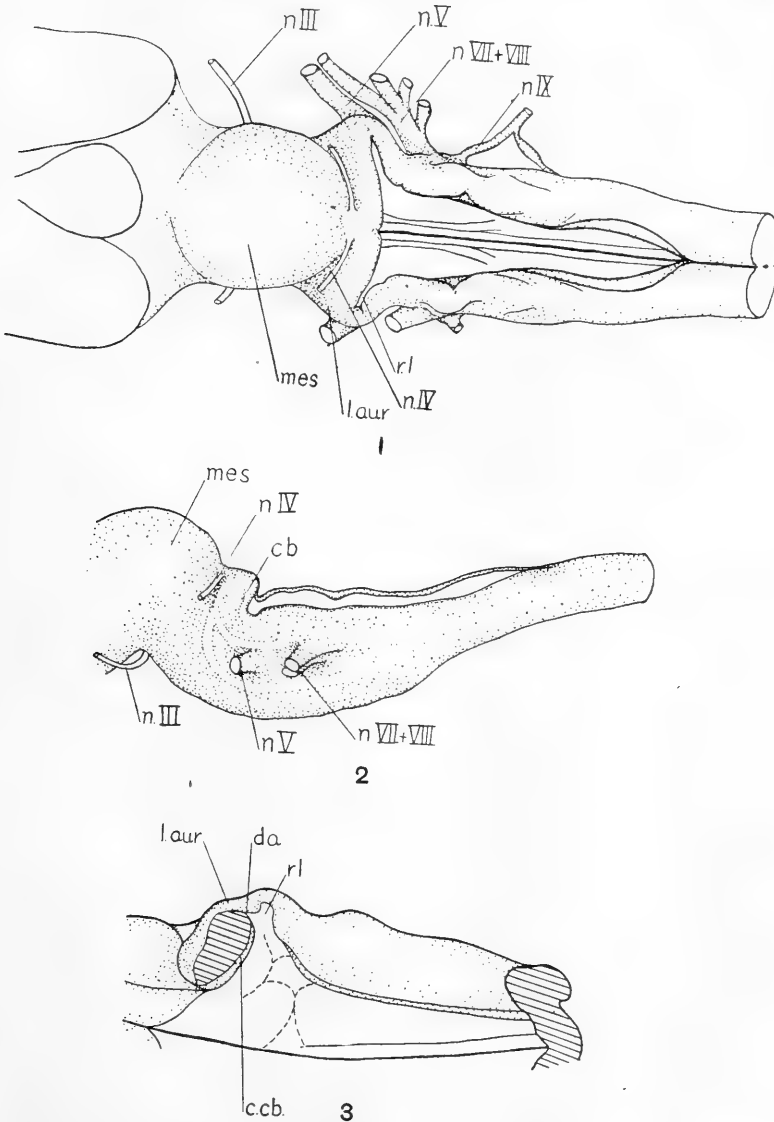


Fig. 1 Dorsal view of mesencephalon, cerebellum, and medulla oblongata of *Amblystoma punctatum*.

Fig. 2 Same as figure 1, viewed from the left side.

Fig. 3 Right half of rhombencephalon of *Amblystoma punctatum*, from which part of the cerebellum has been removed so as to expose the corpus cerebelli and the recessus lateralis. Dorsal view.

Histological structure

Histologically, the cerebellum of *Amblystoma* consists of three layers which may be distinguished by the size of their cells and the relative abundance of their nerve fibers. Two of these layers were recognized long ago by Stieda ('75) in the larval form of *Amblystoma* (Siredon). He considered them to correspond to the granular and the molecular layers, respectively, of the cerebellum of higher vertebrates. This is in agreement with the



Fig. 4 Horizontal section through part of cerebellum of *Amblystoma tigrinum*. Series CLXXXV, sl. 2, sect. 29. Cajal method. $\times 125$.

findings of the present writer. To these two should be added in adult *Amblystoma* a third layer which consists of Purkinje cells. This layer occupies a position between the granular and the molecular layers (fig. 4, *str.Pur.*) and is composed of relatively large cells which resemble Purkinje cells of somewhat reduced type.

These layers correspond in a general way to those of the mammalian cerebellum, not considering the medullary layer of white matter, and will be designated as molecular layer, Purkinje cell

layer and granular layer, respectively. There are important differences of detail, as compared with the corresponding layers of the higher vertebrates, which will be set forth in the description of the different zones.

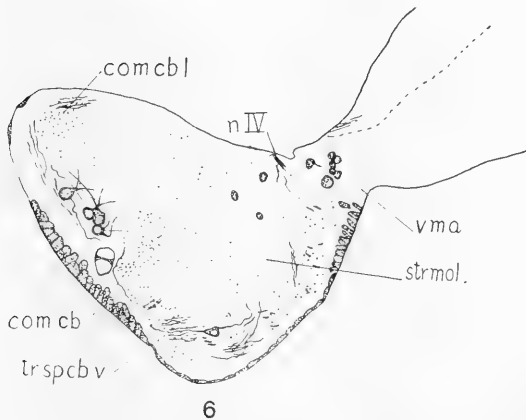
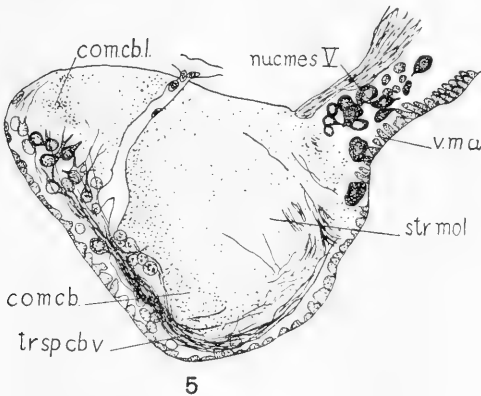


Fig. 5 Sagittal section through cerebellum of *Amblystoma tigrinum* near median plane. Series CLXXXVI, sl. 5. Cajal method. $\times 125$.

Fig. 6 Sagittal section through cerebellum of *Amblystoma tigrinum*. Most medial section but one. Series CLXXXVI. Cajal method. $\times 125$.

The outermost or molecular layer (figs. 4, 5, 6, 7, *str.mol.*), which Stieda described as being composed of a granular ground substance with but few nuclei, consists chiefly of fibers with but few cells. The larger fibers are myelinated, but Cajal sections reveal a

much larger number of fine unmyelinated fibers and their terminal branches. Most of the fibers of this layer are scattered without definite arrangement, but a few definite bundles are present, especially in the lateral portions of the cerebellum. These bundles are located posteriorly and near the layer of Purkinje cells, next to be described. Only two of them could be followed with any degree of certainty as to their relationship. These were the dorsal portion of the cerebellar commissure and the lateral cerebellar commissure (figs. 5, 6, and 15, *com.cb.*, *com.cb.l.*). The few cells present in this layer (fig. 7) are irregularly scattered and of medium size. They are multipolar, but so far as the preparations available indicate, bear no resemblance to the basket cells of the corresponding layer in the mammalian cerebellum. There is a closer similarity to the superficial stellate cells, both in the arrangement of their processes and in the position of the cells near the surface of the molecular layer.

The layer of Purkinje cells is represented in *Amblystoma* by cells of relatively large size. They are arranged in a fairly uniform layer from one to three cells deep (fig. 4. *str.Pur.*). Their dendritic processes, of which from one to three or four may be counted, extend outward into the molecular layer. The branching of these processes is very simple and limited. As revealed by Golgi preparations (figs. 8, 9, 10, and 11), each of the primary dendrites may give off two or three secondary branches, and these in turn may ramify, but beyond this no divisions were observed. In some of the preparations gemmules are present on the secondary and tertiary branches. The primary branches are distributed both horizontally and dorsoventrally, i.e., their distribution is not confined to a narrow zone of relatively wide area, but rather they radiate in such a manner as to outline roughly a pyramid, with the cell body at its apex. The Purkinje cells of this layer are especially numerous in the lateral portions of the cerebellum (*corpora cerebelli*). Some of their processes from this region extend forward and laterally toward the tectum of the midbrain, and some of the smaller branches appear to enter the tectum (figs. 10 and 11).

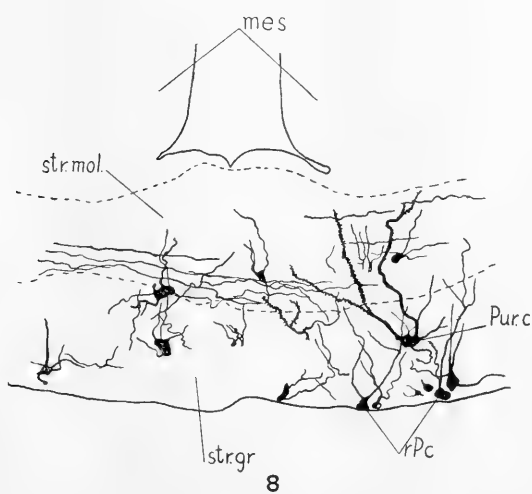
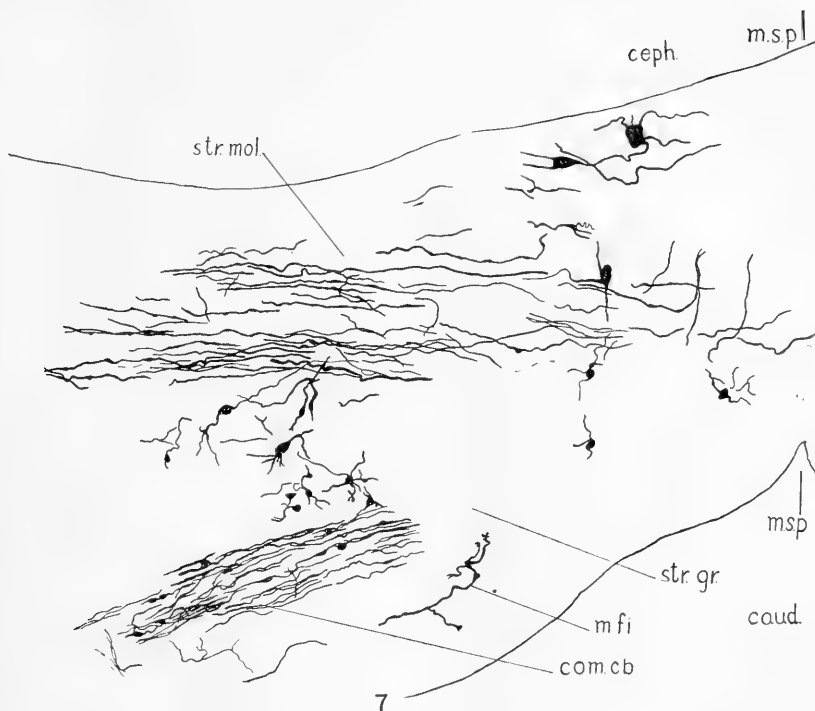
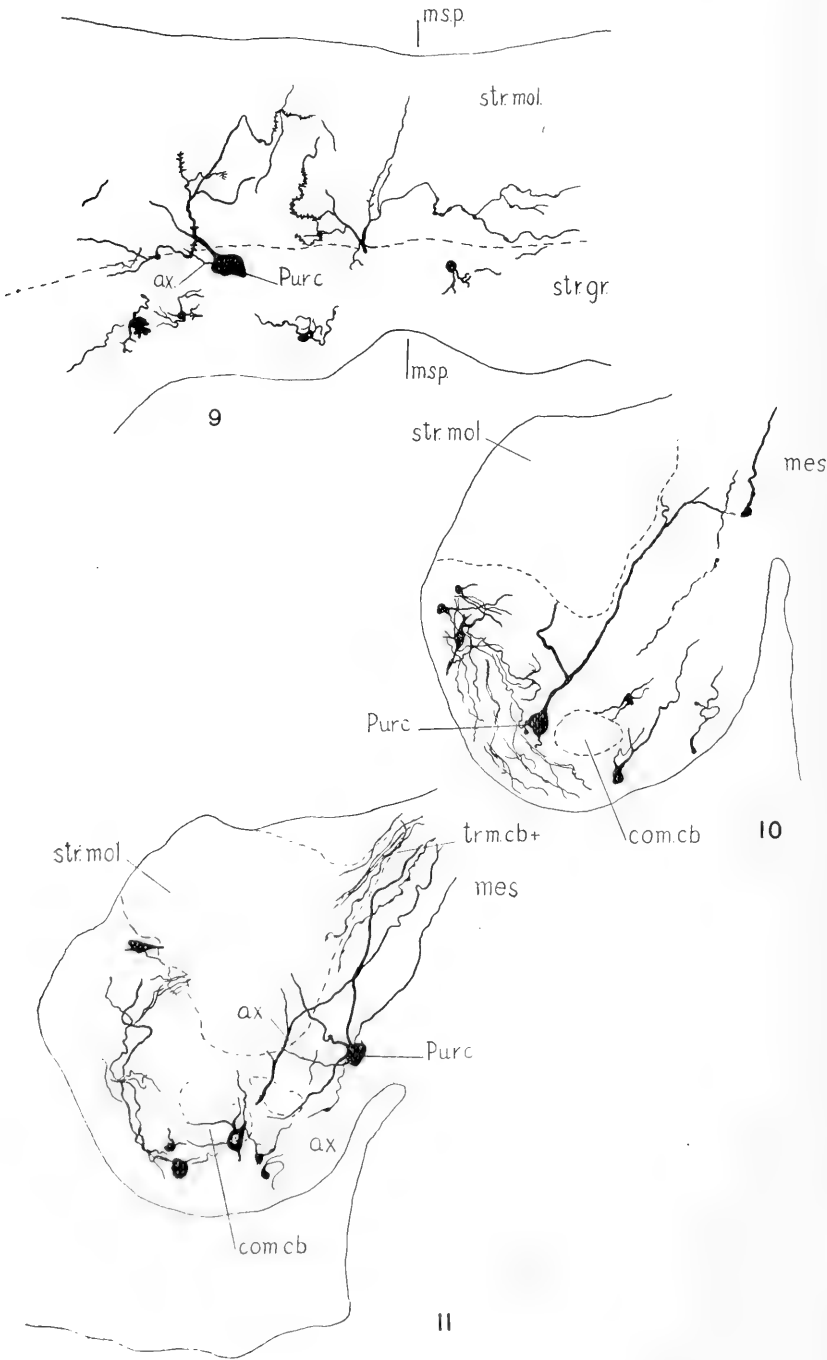


Fig. 7 Horizontal section through the cerebellum of *Amblystoma tigrinum*, showing typical fibers and cells in the molecular layer, and granule cells and a moss fiber in the granular layer. Series CCIV, sl. 3. Golgi method. $\times 196$.

Fig. 8 Horizontal section through cerebellum of *Amblystoma tigrinum*, showing a Purkinje cell, several reduced Purkinje cells, and several other nerve cells. Series CXVII, sl. 3, sect. 1. Golgi method. $\times 60$.



The axones of the Purkinje cells (figs. 9, 11, and 14) have their origin either from the cell body or, more commonly, from one of the primary dendrites close to the perikaryon. They pass into the molecular layer where they become lost among the numerous fibers there present. From many of the cells axone-like processes pass into the granular layer, but turn upward to enter the molecular zone, in the majority of cases observed. A similar passing of the axone of the Purkinje cells into the molecular layer was found by Johnston ('01) in *Acipenser*.

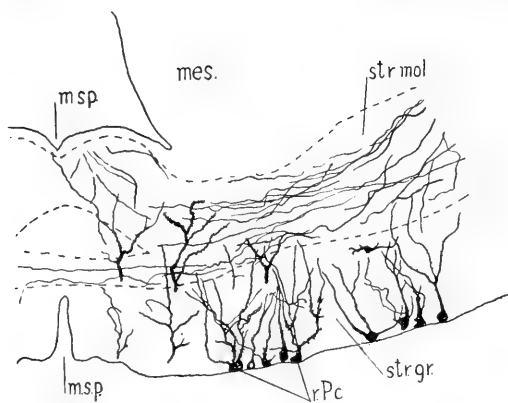
Other cells of somewhat smaller size were observed in Golgi preparations along the posteroventral border of the cerebellum and at various levels within the granular layer (figs. 8, 10, 12, and 13, *r.P.c.*). Typically the cell body is elongated, giving a pear-like form to the cell, but some of fusiform outline were observed. The dendrites extend toward the molecular layer and appear to terminate for the most part within the more posterior and ventral region of this zone, although many do not reach the molecular layer, but end within the granular layer. Some of the processes are studded with gemmule-like projections (figs. 12 and 13).

From the position of most of the cells of this type, along the border of the cerebellum, they might be considered as ependymal cells, related to the peculiarly elongated type characteristic of the cerebellum of other vertebrates. In their morphological characteristics, however, they resemble nerve cells. The dendritic branches do not have the arrangement or appearance of the long processes of the ependymal cells. In connection with many, an axone-like process was observed. This is of simple type, without profuse branching near the cell body, and extends into the molecular layer. In some respects these cells resemble

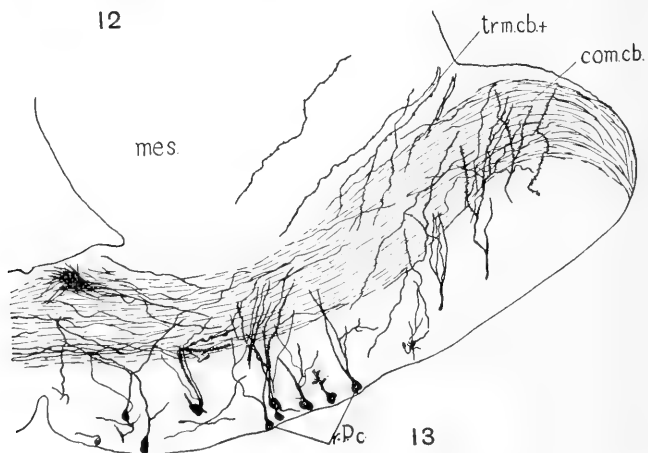
Fig. 9 Horizontal section through cerebellum of *Amblystoma tigrinum*, showing Purkinje cell and granule cells. Series CCIV, sl. 3, sect. 84. Golgi method. $\times 196$.

Fig. 10 Sagittal section through cerebellum of *Amblystoma tigrinum*. Series CCV, sl. 1, sect. 39. Golgi method. $\times 125$.

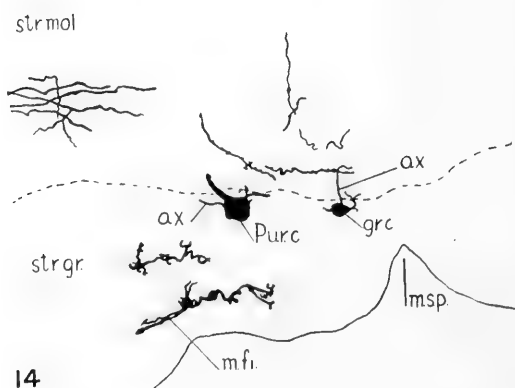
Fig. 11 Sagittal section through cerebellum of *Amblystoma tigrinum*. Series CCV, sl. 1, sect. 30. Golgi method. $\times 125$.



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the Golgi cells of Type II, but the sparsely branched axone would exclude them from this group.

In position and size as well as in general characteristics, these cells appear to correspond more closely than do the larger ones first described to the reduced Purkinje cells found by Herrick in *Necturus*. Various transitional stages between these reduced cells and the more highly differentiated ones were observed. The degree of morphological differentiation appears to correspond roughly with the position of the cell. Those located at the cerebellar border are the most simple in type; cells at various levels in the granular layer appear to be somewhat more advanced, while the cells composing the Purkinje cell layer most closely resemble Purkinje cells of higher forms (figs. 8 to 13). Not all preparations revealed the presence of these reduced cells, but this was probably due to the idiosyncrasy of the Golgi technique. When present in a given series of sections they were more numerous than the larger cells more deeply placed. They were found in greatest number along the cerebellar border about midway between the median line and the lateral border of the cerebellum, but many were observed quite close to the median plane and also laterally, and at various levels between the cerebellar border and the Purkinje cell layer. They might be considered to belong to the granular layer of the cerebellum, but because of their apparent relation to the cells of the Purkinje cell layer they are described at this point.

The granular layer, in addition to the reduced Purkinje cells just noted, consists of numerous small rounded cells, among which are interspersed myelinated fibers. In Golgi preparations these cells are seen to possess several relatively short, tortuous processes which give a stellate appearance to the cell. Many of these

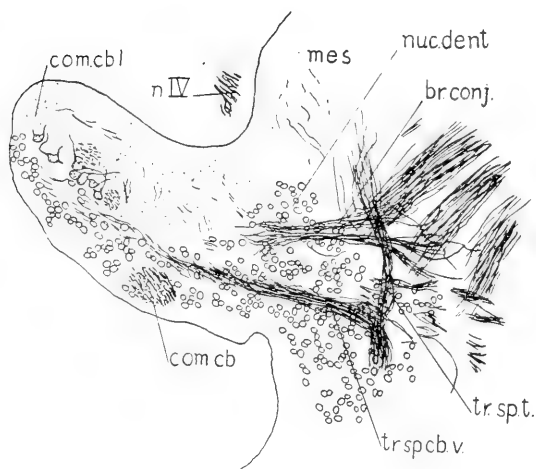
Fig. 12 Horizontal section through cerebellum of *Amblystoma tigrinum*. Series CXVII, sl. 3, sect. 2. Golgi method. $\times 60$.

Fig. 13 Horizontal section through cerebellum, deeper than that of figure 12. Series CXVII, sl. 3. Golgi method. $\times 60$.

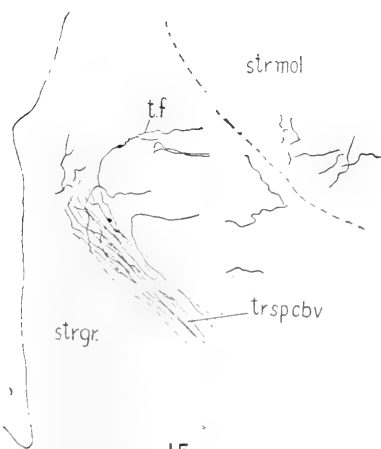
Fig. 14 Horizontal section through cerebellum of *Amblystoma tigrinum*, showing various elements of granular layer. Series CCIV, sl. 3, sect. 83. Golgi method. $\times 196$.

processes terminate in short twigs which resemble the claw-like telodendrites of the granule cells of more highly developed cerebella (figs. 7 to 14). In only a few instances was an axone observed to pass from such cells (figs. 11 and 14). When present it is directed toward the molecular layer, but becomes lost among the numerous fibers of fine caliber there present. No certain indication of bifurcation within the molecular zone was noted.

The fibers of the granular layer appear to be all myelinated, except for the terminal branches which end within it. A well-marked bundle of myelinated fibers passes through the layer from one side of the cerebellum to the other. This includes the cerebellar commissure and, for part of its course, the tractus spinocerebellaris ventralis, although the latter is independent throughout much of its course within the cerebellum (fig. 16, *tr.sp.cb.v.*). Fibers from this large bundle are given off at short, irregular intervals and terminate among the cells of the granular layer. Some of these fibers (figs. 7 and 14, *m.fl.*) appear to be related to the moss fibers of the more highly developed cerebellum. In Golgi preparations they are seen to terminate in short, stout twigs, and along their course, especially at the points of branching, are found the nodosities characteristic of moss fibers. Other terminations within the granular layer consist of long slender processes (fig. 15, *t.f.*) with fine varicosities here and there along their course, but without the stout terminal twigs just noted. These appear to come from the tractus spinocerebellaris ventralis, but this was not determined with certainty. According to Ramón y Cajal, the moss fibers in mammals are the terminal arborizations of afferent fibers which enter the cerebellum through the inferior peduncle, while the climbing fibers, which the last described type of ending most closely resembles, enter from the brachium pontis. It is difficult to apply this statement to the cerebellum of *Amblystoma*, as the brachium pontis is altogether lacking. The moss fibers may be the terminal arborizations of the tractus spinocerebellaris dorsalis, thus passing into the cerebellum through the region which in the more differentiated cerebellum is the inferior peduncle. If the second type described are terminal fibers of the tractus spinocerebellaris ventralis, as they ap-



16



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Fig. 15 Sagittal section through cerebellum of *Amblystoma tigrinum*, showing terminal fibers within granular layer. Series CCV, sl. 1. Golgi method. $\times 196$.

Fig. 16 Reconstruction from five sagittal sections through lateral part of cerebellum of *Amblystoma tigrinum*. The outline and the position of the cells were drawn from section 26 of slide 4, series CLXXXIV, the fibers were drawn in from sections 26 to 30. Cajal method. $\times 60$.

pear to be, they enter the cerebellum through the region which becomes in higher forms the superior peduncle.

Fibers are present everywhere in this layer, from one side to the other of the cerebellum. In the more medial region many run anteroposteriorly (fig. 5) rather than transversely, as is the case more laterally in the organ. Close to the median line most of the longitudinal fibers, as seen in sagittal sections, disappear, and the majority of fibers present are cut transversely. These belong for the most part to the cerebellar commissure, but some are fibers of the tractus spinocerebellaris ventralis.

The granule cells are entirely absent in the median plane (fig. 6), but appear as a few scattered cells very close on either side of this plane. The granular layer therefore increases in thickness laterally, from a very thin zone containing only a few fibers in the median plane, to a layer which occupies nearly half of the thickness of the lateral portion of the cerebellum. In the last-named region it consists, in addition to numerous fibers, of from twelve to fifteen layers of cells. The few cells present near the median line are located in the posterodorsal portion (fig. 5) of the cerebellum. As the corpus cerebelli is approached, the granule cells not only increase in number, as above noted, but also extend toward the more ventral portion of the cerebellum, until this becomes the thickest portion of the granular layer. This cell layer is continuous with the gray matter of the medulla oblongata.

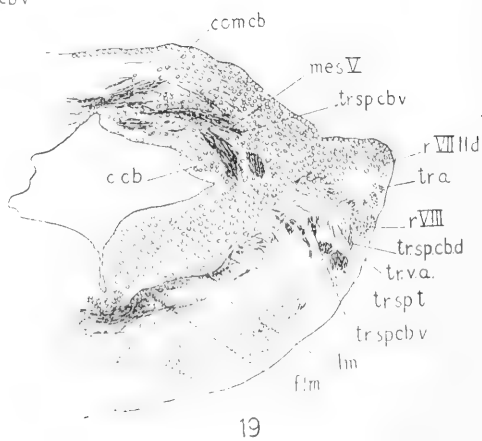
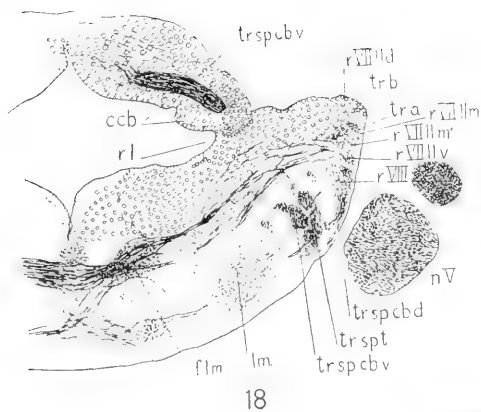
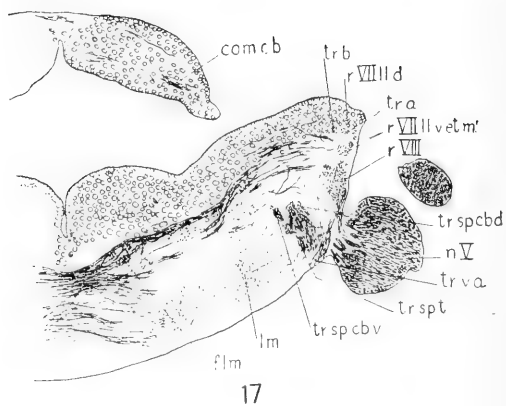
In each lateral half of the cerebellum there is present an enlargement of this gray matter which, from its position and from the origin within it of fibers of the brachium conjunctivum, appears to represent the primitive nucleus dentatus (fig. 16, *nuc. dent.*) already noted in the description of the external form of the cerebellum.

The fiber tract systems of the cerebellum of *Amblystoma* correspond in general with those of *Necturus*, but with some modifications. The efferent fibers consist of internal arcuate fibers which pass forward and downward into the medulla oblongata and into the midbrain. These are chiefly unmyelinated. They evidently represent the cerebellotegmental system. Only in the

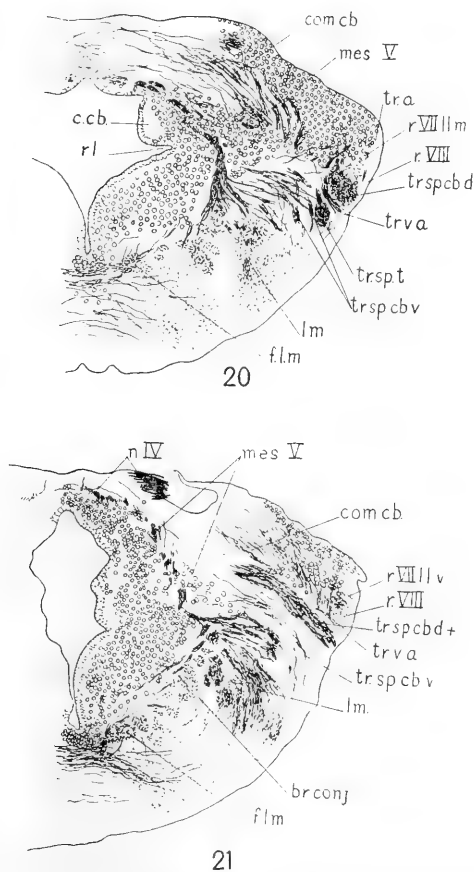
case of those passing forward into the midbrain is there any definite grouping of fibers to form a bundle. This bundle (fig. 16, *br.conj.*) is loosely arranged and has relatively few myelinated fibers. Those present are of small diameter. The unmyelinated fibers are more numerous, so that the bundle is more evident in favorable Cajal preparations than in those stained by the Weigert method. The majority of fibers which enter into this bundle appear to arise from the cells composing the group which has been described as the primitive nucleus dentatus. Some appear to come from the molecular layer, but could not be traced for any distance within this layer. These may possibly represent axones of the Purkinje cells, which, as described above, pass into the molecular zone to be lost within it among the numerous fibers there present. No other efferent cerebellar tracts were recognized, if present.

The afferent fibers which terminate within the cerebellum are definitely grouped into tracts of greater or less size. They are for the most part composed of myelinated fibers.

The tractus spinocerebellaris ventralis is the largest of the afferent tracts. It lies dorsally and laterally of the bulbar lemniscus, and is closely associated, as pointed out by Herrick in both *Necturus* and larval *Amblystoma*, with the tractus spinotectalis. Just before reaching the level of the superficial origin of the V nerve, the two tracts separate to some extent, but not entirely. They continue forward as far as the ventral and anterior portion of the corpus cerebelli (fig. 16, *tr.sp.cb.v.*, *tr.sp.t.*). Here the ventral spinocerebellar tract is completely separated from the mixed bundle and turns dorsally and medially to pass into the body of the cerebellum (figs. 16 and 21, *tr.sp.cb.v.*). Its fibers remain distinct from those of the cerebellar commissure for some distance, then it becomes part of the commissural bundle; but in sagittal sections the tract, somewhat reduced in size, is seen to separate again from the commissure on its dorsal side (fig. 16). Its fibers pass among the cells of the granular layer a few at a time as the median plane is approached, but many cross to the opposite side of the cerebellum. The bundle is therefore not greatly reduced in size as it also receives fibers from the corresponding tract of the opposite side.



The tractus spinocerebellaris dorsalis offers considerable difficulty in identification. As seen in Weigert preparations (figs. 17 to 21, *tr.sp.cb.d.*), a bundle of myelinated fibers emerges from the cerebellar commissure near the ventrolateral extremity of the latter and assumes an independent course ventrally and caudally.



Figs. 17 to 21 Five transverse sections through medulla oblongata and cerebellum of *Amblystoma tigrinum*, arranged in series from the level of the superficial origin of the V nerve to the level of the superficial origin of the IV nerve, showing the arrangement of the fiber tracts of the cerebellum and some of the tracts of the medulla oblongata for orientation. Series IIC, sects. 574 (fig. 21), 585 (fig. 20), 595 (fig. 19), 603 (fig. 18), and 605 (fig. 17). Weigert method. $\times 28$.

Within the commissure the bundle is distinguishable for a short distance toward the median plane by reason of the smaller diameter of its myelin sheaths, but it soon becomes lost among the numerous fibers of various size which compose the commissure. Caudad this bundle runs mesial to the VIII tract, but before reaching the superficial root of the V nerve, it turns ventrally to pass beneath the latter. Cephalad of this point, however, it becomes so intermingled with other fibers as to be very difficult to follow. It takes its further course ventrally of the spinal V tract for a short distance, but before reaching the level of the VIII root it becomes lost among other fiber bundles.

Some of the fibers of the mixed bundle cephalad of the V root pass as far caudally as the superficial origin of the trigeminus. At this point many of them pass ventrally just anterior to the V root fibers, others find their way ventrally through the V root bundle, and the two groups reunite below the spinal V tract. These constitute the ascending gustatory tract (figs. 17 to 21, *tr.v.a.*). Cephalad this tract passes through the auricular lobe, and appears to come into relation with a group of cells just rostrad of the lobe, but which are evidently a part of the mid-brain. In adult *Amblystoma* this group of cells is more obscure than in the larval form, in which they stand out very clearly. The ascending gustatory tract does not appear to have any direct connection with the cerebellum.

A small bundle of myelinated fibers of reduced diameter from the trigeminus shows bifurcation of the fibers just before the nerve enters the medulla oblongata, or just within the latter. One branch of these bifurcated fibers may be observed in Golgi and Weigert sections to take its course ventrally of the VIII tract to the auricular lobe, but some of these fibers appear to pass into the cerebellar commissure in company with the dorsal spinocerebellar tract. The other branch of the bifurcated fiber passes posteriorly into the medulla and possibly into the cord. This is the only indication of trigeminal fibers which appears to terminate within the cerebellum.

In Golgi sections cut sagittally a few fibers were observed to pass between the pars dorsalis hypothalami and the lateral por-

tion of the cerebellum. The cells of origin appear to lie in the pars dorsalis hypothalami. The fibers pass from this region dorsocaudad and toward the midplane. After passing dorsal to the region of the interpeduncular nucleus, they dip ventrally behind this nucleus, then assume a more dorsal course and pass through the midbrain into the tegmentum and the cerebellum. Within the cerebellum (figs. 11 and 13, *tr.m.cb.*) they are scattered loosely in the dorsolateral region, and show characteristic varicosities. They appear to come into relation with dendritic processes of the larger Purkinje cells (fig. 11) above described, which extend toward the midbrain. These fibers correspond to the tractus mammillocerebellaris which appears to be present in *Necturus* also. From the interpeduncular region, in *Amblystoma*, a number of fibers accompanies the mammillocerebellar tract in its course toward the cerebellum, lying ventral and parallel to it. The fibers appear to have their origin from cells in the interpeduncular nucleus. They pass dorsally and caudad into the tectum mesencephali, where many of them appear to end. Others, however, appear to continue caudad into the cerebellum in company with those of the tractus mammillocerebellaris. This appearance was chiefly due to the greater numbers of fibers in the region of the cerebellum occupied by the tractus mammillocerebellaris, as compared with the number constituting this tract rostrad of the nucleus interpeduncularis. The number within the cerebellum appears to correspond more closely with that of the combined tracts in their course between the interpeduncular region and the mesencephalon, but this greater number within the cerebellum may be due to profuse terminal branching of the mammillocerebellar fibers. This point could not be determined in the material available. It is possible that some are fibers of the secondary gustatory tract which pass through the lateral region of the cerebellum. Herick ('17) believes that the mammillocerebellar tract which he had previously tentatively established in his description of the cerebellum of *Necturus* "includes the combined secondary and tertiary visceral tract, with perhaps mamillotegmental fibers mingled with them." In the Golgi preparations of *Amblystoma*,

it appears quite evident that some of the fibers of the combined tract terminate within the cerebellum, so that the designation, *tractus mamillocerebellaris*, is employed.

The mesencephalic V tract (figs. 19 to 21, *mes.V*) has not been studied by the writer except in its relation to the cerebellum. It is composed of coarse myelinated fibers which form a well-defined bundle of characteristic appearance. This bundle passes dorsorostrally and mesad from its origin at the superficial roots of the trigeminus toward the mesencephalon. In its course it traverses the cerebellum and for a short distance is more or less intermingled with the cerebellar commissure (fig. 19). The fibers of the mesencephalic V tract, however, may be distinguished from those of the commissure. They merely pass through the latter in their course between the midbrain and V nerve. In the boundary region between the cerebellum and the tectum, however, the mesencephalic V fibers become intermingled with others, especially those of the IV nerve, in such a manner that they are difficult to follow in Weigert preparations. Their further course has not been studied. So far as observed, they have no immediate functional relation to the cerebellum. The trigeminal fibers previously noted which appear to pass into the cerebellum have no relation, so far as could be determined, to the mesencephalic V tract.

There are some indications of tectocerebellar fibers, but they do not form a distinct tract and are so intermingled with other fibers in this region that a clear analysis was not possible.

The fiber tracts which pass into the auricular lobe have much the same relationship in adult *Amblystoma* as they have in the larval form, so far as the available material indicated.

The *VIII tract* forms a well-defined bundle of fibers which lies close to the lateral margin of the area acustica. From its origin at the level of the VIII nerve to a point slightly rostrad of the V root, two bundles, a dorsal and a ventral, may be observed in adult *Amblystoma*. This is the condition observed by Herrick in the larval form, except that the two tracts are distinct only as far as the superficial origin of the V root in the latter. The fibers continue forward from this point as a single tract which

passes into the lobus auricularis. Within the auricular lobe fibers pass dorsally and mesad from the main bundle (figs. 17 to 21, *r.VIII*). Most of these disappear among the cells and fibers of the auricular lobe, but many appear to pass into the corpus cerebelli. The VIII tract continues forward, diminished in size, to the most rostral region of the auricular lobe, where its fibers disappear in a manner similar to those in the more caudal portion of the lobe.

The lateral-line roots of the VII nerve can be followed as small bundles of myelinated fibers forward into the auricular lobe. Four distinct bundles can be recognized in the adult Amblystoma at most of the levels between the superficial roots of the facialis and the auricular lobe. These apparently are the continuation of the four roots of the lateral-line component of the VII nerve described by Coghill ('02). The three more dorsally located (figs. 17 to 20, *r.VII l.l.d.*, *r.VII l.l.m.*, and *r.VII l.l.m'*.) could not be followed with certainty beyond the posterior region of the auricular lobe, but the ventral tract (fig. 21, *r.VII l.l.v.*) continues dorsal to the VIII tract to terminate in the rostral end of the auricular lobe.

SUMMARY

The cerebellum of Amblystoma has the general characteristics of this organ as described in other urodeles, but it shows some advances of structure and organization not present in the lower forms of this group of vertebrates.

The more important of these advances are: 1) increased size of the corpus cerebelli; 2) the presence in the corpus cerebelli of a group of cells which appear to foreshadow the nucleus dentatus; 3) the presence of a definite zone of Purkinje cells, the cells of which have the general characteristics of this type of neurone as present in higher vertebrates; 4) the presence of granule cells and moss fibers within the substantia grisea, which corresponds to the stratum granulare of higher forms.

The principal fiber-tract connections, with modifications of detail, are similar to those of lower urodeles. These include the tractus spinocerebellaris ventralis, the tractus spinocerebellaris

dorsalis, and the tractus mammillocerebellaris, on the afferent side. To these, in *Amblystoma*, should be added some fibers from the trigeminus and also fibers of the VIII tract which pass into the body of the cerebellum from the auricular lobe. There are evidences of a tectocerebellar tract, but the fibers are diffuse and are intermingled with other fibers in the region of the entrance into the brain of the IV nerve in such a manner as to be difficult of separation from the others.

The efferent fibers include the brachium conjunctivum and numerous arcuate fibers which are not aggregated into definite tracts, but which appear to correspond to the cerebellotegmental system.

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Resumen por los autores, Swale Vincent y A. T. Cameron.
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Nota sobre un reflejo inhibitor de la respiración en la rana y
otros animales.

En todos los animales existen mecanismos reflejos que inhiben los movimientos respiratorios cuando la cabeza se sumerge en el agua. Los receptores de tales reflejos están situados probablemente en el epitelio de la membrana mucosa nasal. En adición los autores han encontrado algunas pruebas que demuestran que en la rana existe un mecanismo reflejo accesorio que depende de la oclusión de los orificios nasales externos y la cesación de la corriente de aire en los pasajes respiratorios. Ambos reflejos pueden entrar en acción cuando la rana está sumergida.

Translation by José F. Nonidez
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A NOTE ON AN INHIBITORY RESPIRATORY REFLEX IN THE FROG AND SOME OTHER ANIMALS

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Some of the results stated for the frog in the present communication were read to the Scientific Club of Winnipeg on February 24, 1914. At that time we were not acquainted with any previous account of the phenomenon, but subsequently discovered Axenfeld's paper,¹ published in 1911. This appears to be a preliminary communication and contains no references to literature. We have failed to find any subsequent communication by Axenfeld or any other author upon this reflex in the frog, and since Axenfeld does not appear to state fully the facts of the case, we have decided to make a brief communication at the present time.

Moreover, the observations on other animals made by several previous observers seem to have been overlooked by the most recent writers. Starling, writing in Schäfer's Text-book,¹⁵ says:

A pure expiratory reflex may also be brought about by gentle stimulation of the nasal mucous membrane of the rabbit, as by application of chloroform vapor. A similar expiratory pause is caused in many animals by dipping the nose into water, or even by plunging the lower half of the body into water (Tauchreflex). The temperature of the water is of no influence on the results of the experiment. Frédéricq⁹ has shown that a specially long expiratory pause may be produced in a diving animal, such as the duck, by allowing a stream of water to flow on its beak. The teleological importance of these reflex cessations of respiration, which have been classed together by Miescher-Rüsch¹³ as apnoea spuria is obvious.

We have found other references to the authors here quoted, including a paper by Foà⁸ modifying Miescher's classification of apnoeal reflexes. We have, unfortunately, not been able to

consult the original papers of these authors, and from the references we cannot be certain how far they have dealt with the precise points with which we are most concerned.

A. THE FROG

The normal respiration of the frog has been fairly completely studied. A summary of the known facts is given by Baglioni.³ It is sufficient to remark here that a large share in the function of respiration is borne by the skin, the lungs providing an accessory mechanism consisting of two separate movements, *a*) that of the mouth with the lungs closed off and, *b*) the true lung movements, the latter only occurring at certain intervals. The movements with which we are concerned in this paper are those occurring in the throat and nostrils. (For an account of the normal respiration compare also Willem.¹⁶)

Two different kinds of external influences have been described as affecting the movements in question. Graham Brown,⁶ who gives a good account of the literature, deals chiefly with the influence of the nervous system and the labyrinth, but mentions certain external factors producing inhibition, such as shaking of the animal, a blow on the nose, stimulation of the skin, etc. He does not mention the effect of immersion in water or of plugging the nostrils. Axenfeld,¹ who seems to have been the first to describe specifically the immersion apnoea in the frog, attributes the phenomenon to a definite stimulation of the nerve endings of the nasal mucous membrane by means of water. Some of the earlier observers referred to by Graham Brown describe an apnoeal reflex in the frog due to various afferent impulses arising from different parts of the body surface.

The immersion apnoea is one which must have been familiar to naturalists for a long time. The moment that a frog becomes completely immersed in water, the respiratory movements cease, and remain in abeyance as long as the animal continues to be immersed. The most elementary observation shows that cessation of breathing occurs at the moment that both nostrils touch the water. Our observations were directed chiefly toward determining the nature of the stimulus which inhibits the respiratory movements.

Axenfeld comes to the conclusion that the nasal mucous membrane is stimulated specifically by air and by water, and that the stimulation calls forth in one case movements of breathing, and in the other inhibition of these. He states that 20 per cent acetic acid destroys the reflex by damaging the mucous membrane of the nostril; if a frog is immersed after such treatment it continues to breathe, filling its mouth with water.

He also states that the inhibitory reflex is not altered after section of the first division of the fifth nerve with its nasal branch.

We have carried out the following series of experiments:

1. Several frogs were treated with 20 per cent acetic acid, following Axenfeld's directions, and using his precaution of plugging the mouth with absorbent cotton while the nostrils were being treated with the acid, in order to prevent more extensive damage. We found that the proceeding interferes with the normal respiration. The animal can no longer breathe properly, even in air. When such an animal is placed in water it is true that it continues the movements of respiration, but this is rendered possible by the opening of the mouth to some extent and not through true nasal breathing. The interference with breathing in the air is probably due to swelling of the epithelium and excess of mucus in the nasal cavity, and in this case also the animal breathes through the mouth.

We have repeatedly observed that during free-air breathing when through any cause the nasal passages become obstructed, after a while the animal will continue breathing by occasionally opening and closing the mouth.

2. More complete destruction of the epithelium of the nasal passage can be produced by the actual cautery. We have done this with several frogs, and if the passage of the nose be kept free something approaching a regular respiration will go on for some time. This, however, ceases instantly on immersing the animal in water. It has been pointed out to us that the cautery will not destroy the epithelium of the deep recesses of the nasal cavity.

3. Early in our experiments it was noticed that plugging the nostrils with blunt seekers or by placing the fingers over the

nasal apertures, immediately stops the movements of the floor of the mouth. The inhibition is temporary, and lasts from eight to fifteen seconds. Mechanical stimulation of the interior of the nostril does not produce this effect, nor does mechanical stimulation of the skin in the neighborhood of the nostril. A weak electrical stimulation in the same neighborhood produces no effect, but a strong stimulation with induced current causes the animal to throw back its head, and this type of action, as observed by Graham Brown and others, checks the respiratory movements. But it is interesting to note that when the head is thrown back or pushed back by the hand, in either case there occurs closure of the nostrils concurrently with cessation of respiration, and stimulation of the skin of the back will induce throwing back of the head, closing of the external nares, and stoppage of respiration.

These changes in some ways simulate the posture reflex observed in the duck by Huxley (see below). It should therefore be pointed out here that when a frog is immersed in water or when the nostrils are plugged, no such change of posture need necessarily occur, and does not usually so occur, so that the reflex is not due to this cause.

4. The following experiment was carried out with two frogs: Fine, accurately fitting cannulae were inserted into the nostrils after cauterizing. The animal was then immersed with the extremities of the cannulae communicating with air; so long as the cannulae were not plugged by mucus, and did not by pressure mechanically occlude the nasal passages (these errors were specifically guarded against in the cases observed), respiration went on normally, and no inhibition could be induced such as those described as similar to the postural reflex (it being no longer possible to close the nares). On removing such tubes from the nostrils while the frog was under water, breathing stopped immediately.

Repetition of this experiment with a number of other frogs gave less satisfactory results, owing to the difficulty of keeping the cannulae free from mucus, etc. In some cases where the cautery had enlarged the aperture of the nostril considerably so

that the cannulae did not completely occlude it, immersion of the animal, snout-end last, caused immediate cessation of respiratory movements, apparently through the entry of water into the nostrils round the outside of the cannulae.

In all the above experiments similar effects were produced by immersion in water, whether the animal was immersed snout first or snout last, except in cases where the mucous membrane of the nostrils had been damaged, when change of posture produced an effect in some animals, this being almost certainly due to increased or decreased plugging of the nostrils with mucus.

5. Cannulae were inserted into the nostrils of an intact animal; the respiratory movements were seriously affected, but after some minutes an imperfect kind of respiration began. The free ends of the cannulae were then immersed in water, and after several seconds the same imperfect respiratory movements recommenced, although only water could be taken in. This occurred even when the nostrils were also submerged.

All these experiments were carried out with *R. pipiens* from Illinois.

Conclusions

Apparently Axenfeld is right in supposing that the most important factor in the submersion stoppage of respiratory movements is a specific stimulation of the nasal mucous membrane by contact with water. This is supported by our experiments nos. 4 and 5, and is not definitely contradicted by no. 2. It may be observed in this place that this inhibition of the respiratory movements is more pronounced and permanent in the frog than in other classes of animals, since, as shown by experiments carried out in this laboratory,⁷ the animal can live for many weeks under water, and during this time makes no attempt at respiratory movements, the floor of the mouth remaining permanently in the expiratory position.

In addition to the reflex described above, there appears to be another and quite separate one, caused by plugging the nostrils (cf. experiment 3). We are tempted to suggest that the sense of resistance brought about by the impeded air flow and experi-

enced through the muscular sense of the throat muscles acts as the afferent stimulus for the reflex.

B. BIRDS

The respiratory reflexes in the duck have been dealt with in a series of papers by Frances M. Huxley.^{10,11,12} This observer, who makes no reference to the work of Frédéricq, noted that respiration in the duck always ceased when the head and neck were immersed in water. In her conclusions she says (¹⁰, p. 152):

Thus submersion of a duck's head gives rise to complete apnoea followed by a compensatory hyperpnoea. Submersion of the end of the bill does not produce this; submersion of the external nares does so only to a certain degree. For its complete production entire immersion of the glottis, the anterior portion of which lies 2.5 cm. behind the posterior border of the external nares, is required.

In her second paper published on the same date she seems to have somewhat altered her opinion. Here she states: "As soon as the mucous membrane of the nostrils, etc., comes in contact with the water, a reflex apnoea is produced" (¹¹, p. 174).

Her detailed description of experiments points to the latter view as being the more correct. Both with immersion of nostrils and immersion of the whole head there was a comparable percentage of cases where one or two respiratory movements were made after immersion.

We have made several immersion experiments with the duck, and we believe complete immersion of the nostrils (and not necessarily of the whole head) is sufficient to induce apnoea.

Doctor Huxley appears to assume that the mechanism is a reflex from the mucous membrane of the nostril, as Axenfeld assumed in the case of the frog, but her experiments did not eliminate the possibility of a mechanical factor, such as we have stated to be efficient in the latter animal. Such a mechanical factor, however, does not appear to be in operation in the case of the duck.

Plugging the nostrils does not interfere with the normal breathing in the duck, but this is partly due to the fact that the

animal breathes through the mouth. Surrounding the mouth cavity with water and subsequently plugging the nostrils, does not interfere with respiratory movements. Further, immersion of the head in water while the nostrils are plugged with the fingers has no effect, although on removing the fingers from the nostrils while the head is still under water all respiratory movements are immediately inhibited. This seems to us conclusive evidence that in the duck a fluid contact with the mucous membrane of the nostril is essential to the reflex.

We can fully confirm Frédéricq's observation that pouring water over both the nostrils will bring about the apnoea, although the mouth is freely exposed to air. Further, we find that a stream of water directed through the nostrils produces the same effect. Plugging one nostril with the finger and directing a stream of air (under pressure) against the other induce the reflex, but this may be due to distention of the air sacs, which produces apnoea, according to Baer.² Stimulation by introducing a solid object into the nostril (such as wires, india-rubber tubing, etc.) is not effective.

It thus appears that the apnoeal reflex in the bird is of a similar nature to the more important one in the frog.

The postural reflex fully described by Huxley and by Paton¹⁴ in the duck may be easily demonstrated, and we have no further observation to offer upon this phenomenon. Whether there is a similar reflex in the frog we cannot yet be sure. What we at first thought to be a postural reflex in that animal seems connected with closure of the nostrils, and is probably something different.

We have carried out a series of experiments upon the pigeon. Immersion of the nostrils in water or direction of a stream of water upon the nostrils immediately stops the respiratory movements. A stream of air under pressure produces the same effect as in the duck, but this may be due to distention of air sacs (see above). On the other hand, plugging the mouth and nostrils and mechanical and electrical stimulation are not effective.

C. MAMMALS

According to Huxley, Beau⁴ in 1860 observed that when a dog is immersed in water it immediately ceases breathing, and this was confirmed by Paul Bert⁵ in 1870. Beau attributed the cessation to reflex action from contact of water with the respiratory orifices, while Bert considered it due to voluntary movement.

We have found that the reflex can be readily demonstrated in the white rat.

We have shown that if the snout of the non-anaesthetized animal be immersed in water at body temperature (to avoid effect of cold), even if part of the mouth remains in contact with air, immediately the nostrils are immersed they are closed and respiration ceases.

Precisely the same occurs with the anaesthetized animal, and the result can easily be recorded by the graphic method. The reflex is very definite, and—with a short immersion lasting a few seconds only—appears to persist for some seconds after removal of the snout from water.

The observation upon the anaesthetized animal is sufficient to negative any suggestion of voluntary action.

Further observations showed that a stream of air under slight pressure stops the respiration, while plugging the nostrils and mouth and mechanical and electrical stimulation do not do so.

The same results are obtained with the anaesthetized rabbit.

Although we are not yet prepared to discuss fully the nature of the reflex in mammals, we think that it is probably of the same nature as that in the duck.

GENERAL SUMMARY

In all vertebrates there appear to be reflex mechanisms which inhibit the respiratory movements when the animal's head is submerged beneath the surface of water. The receptors for these reflexes are probably situated in the epithelium of the nasal mucous membrane. In addition we have adduced some evidence that in the frog there is an accessory reflex mechanism

depending upon the plugging of the nostrils and stoppage of the flow of air through the respiratory passages. Both these may come into play when the frog is submerged.

Doctor Herrick has pointed out to us that there are "four different types of innervation of the nasal region, the functional limitations of no one of which have been clearly shown. These are:

1. Trigeminal nerve endings.
2. Olfactory nerve.
3. The vomeronasal nerve, with the same type of peripheral endings as the olfactory, but limited to the vomeronasal organ (Jacobson's organ) peripherally and with a special part of the olfactory bulb (the *bulbus accessorius*) centrally.
4. The *nervus terminalis*—peripheral endings unknown and central endings independent of the olfactory bulb.

"Ayers (*Jour. Comp. Neur.*, June 15, 1919, vol. 30, 323) has suggested some of the physiological problems here. The terminal nerve is found in all vertebrates from fishes up and is therefore probably not specifically concerned with air breathing. On the other hand, Jacobson's organ and its nerve are well developed in air breathers. Their reduction in birds may account for some of the peculiarities of these animals."

Since we shall find it impossible to pursue this line of investigation any further, we can only hope that the above observations may at any rate serve the purpose of suggesting a field for comparative physiological research.

Some of the experiments above described were carried out by Mr. K. J. Austmann under our direction.

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Resumen por los autores, H. W. Norris y Sally P. Hughes,
Colegio Grinnell.

Los nervios craneales, occipitales y espinales anteriores de
Squalus acanthias.

El presente trabajo es una descripción del origen central, ganglios, trayecto y distribución periférica de los nervios craneales, occipitales, y tres primeros nervios espinales de *Squalus acanthias*, los cuales se comparan con los correspondientes de *Mustelus californicus* e incidentalmente con los de *Raja radiata*. También se incluye una descripción detallada del plexo ciliar del simpático y una descripción mas breve de los ganglios del simpático hallados en los nervios branquiales. Se describe así mismo el origen del nervio hipobranquial (hipogloso) a expensas del nervio occipital y dos primeros nervios espinales. Los elementos sensorios somáticos faltan en los nervios glossofaríngeo y vago. En el glossofaríngeo existe un constituyente lateral provisto de raíces y ganglios distintos. Hay una diferencia marcada entre los nervios constituyentes del complejo del vago. Los órganos sensoriales de la línea lateral y su inervación se revisan brevemente. Existen tres (o cuatro) tipos de órganos sensoriales de la línea lateral, a saber: 1) órganos canaliculares; 2) órganos en forma de fositas; 3) ampollas de Lorenzini; 4) el órgano sensorial espiracular etc, que está probablemente formado por ampollas de Lorenzini modificadas. Se describe la inervación de todos estos órganos.

Translation by José F. Nonidez
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THE CRANIAL, OCCIPITAL, AND ANTERIOR SPINAL NERVES OF THE DOGFISH, *SQUALUS ACANTHIAS*

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FIFTY-THREE FIGURES

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INTRODUCTION

The common dogfishes of Europe and America have long been used in biological laboratories as types introductory to courses in the comparative anatomy of vertebrates, neurology, etc. An exact knowledge of the structure of these forms, particularly of the head, has, strangely enough despite their general use, not yet been gained, except in reference to certain parts. Marion ('05) has described the muscles of the head in *Squalus acanthias*. The skull of the same form has been accurately described and figured by Wells ('17). Neal's ('97, '98, '14, '18) studies on the development of the nervous system, the metamerism of the head, and the innervation of the hypoglossal musculature have thrown much light upon adult conditions. It has long been customary to regard the cranial nerves of the selachians as a pattern to which may be referred the cranial nerves of all other vertebrates. Without doubt, this practice has been justified, but it is a matter of regret that so little has been positively known of the exact composition, origin, and distribution of the main nerves, to say nothing of the smaller divisions.

There is a vast and bewildering literature upon the cranial nerves of the elasmobranch fishes. Much of it is based upon inexact observations, much of it has been published in support of theories that have little basis in fact. Doubtless, our knowledge of cranial anatomy had to be exploited in the interests of now generally abandoned theories of the metamerism of the head. Unquestionably, there is a fundamental segmentation of the

head, but in seeking to establish a theory we have too often overlooked the obvious facts. A review of the extensive investigations into the anatomy of the selachian nervous system would be of little value in the present research, for, notwithstanding the voluminous literature, few attempts have been made in an exact analysis of the cranial nerves of these forms. Strong ('03, '04) published very brief abstracts of very extensive and thorough-going analyses of the cranial-nerve components in *Squalus acanthias*. Landacre ('16) described the embryonic condition of the cranial ganglia and nerve roots in the same species. Houser ('01) made a careful study of the neurones of the selachian brain, using chiefly *Mustelus canis*. Allis' ('01) studies upon *Mustelus laevis* have made valuable contributions to our knowledge of the selachian nervous system, but the writers are unable to follow him in many of his conclusions.

It is in the hope of clarifying our present knowledge of the basic structures that constitute the peripheral portions of the cranial nerves of the elasmobranch fishes that the writers have undertaken an analysis of the composition of these nerves. At the very outset of the presentation of our results, it may be said that there has been throughout the investigation a remarkable clearness as to the certainty of the facts. Long and wearisome tracing out of details has characterized the progress of the research, but there has been a satisfying certainty about all the minutiae that has made the task a pleasant one. Probably in no other vertebrates are the nerve components more distinct histologically than in the selachians. Given the proper technical treatment, there need be little uncertainty about the findings.

For general bibliographies on the cranial nerves of the selachians the reader is referred to the papers of Allis ('89, '97, '01), Cole ('98), and Neal ('98, '14):

The writers are greatly indebted to Prof. H. V. Neal, of Tufts College, for embryos of *Squalus acanthias*, to Prof. J. F. Daniel and Mr. F. H. Ballou, of the University of California, for embryos of *Mustelus californicus*, and to Mr. Fred T. Lane, of Rockport, Massachusetts, whose kindly cooperation has been that of a

friend rather than an agent. For the loan of literature and the freedom of access to his private library the writers are under lasting obligation to Prof. J. S. Kingsley, of the University of Illinois.

Acknowledgment is made of a grant from the Bache Fund of the National Academy of Sciences, which has made possible the early completion of this research.

MATERIAL AND METHODS

Heads of *Squalus acanthias* and of *Mustelus californicus*, in the 'pup' stage, were fixed in vom Rath's picric-acetic-osmic-platinic mixture and sectioned by the celloidin method. The best results were obtained by fixing in a 10 per cent solution of neutral formalin, followed, after thorough washing, by the vom Rath treatment. Where the latter is applied to fresh material (dogfish), many kinds of structures are blackened; but when preceded by formalin fixation, only nerves and muscles are thus affected and stand out distinctly, on a nearly-colorless background. Material long preserved in formalin does not seem to react as favorably as that which has been recently fixed. The sections were cut 15μ and 20μ thick, mounted in balsam on lantern-slide covers, and covered with large sheets of mica. In most instances the sections were counterstained on the slide in Van Gieson's picrofuchsin. By this treatment the most faintly stained nerve fibers could be distinguished readily from connective tissue. Projections of the nerves were made upon the sagittal and horizontal planes. A wax model was made of one half of the medulla oblongata, especial attention being given to an analysis of the ganglia. A model of some of the structures found in the orbit has served to make clear some of the relations of the ciliary ganglia.

THE OLFACTORY NERVE

The writers see no good reason for disagreeing with Brookover ('10, '11, '14, '15) in the opinion that the *nervus terminalis* is in origin and structure related to and a part of the olfactory nerve. Belogolowy ('11), from his researches upon the develop-

ment of the *nervus terminalis* in Selachians (*Acanthias*, *Raja*, *Torpedo*), concludes that the olfactory nerve develops out of and supplants a primary ganglionic nerve of the olfactory vesicle. The *nervus terminalis* of selachians, according to him, is but a persisting remnant of the primary nerve. The primary olfactory nerve is a derivative of the olfactory placode. The secondary olfactory nerve (olfactory nerve proper) is then but a derivative of the *nervus terminalis*. Brookover agrees with Belogolowy, except that he does not see any need or propriety in calling the ganglion a primary olfactory ganglion.

To the account given by Locy ('03) of the *nervus terminalis* of *Squalus acanthias* the writers have nothing of importance to add. In the words of that writer:

Starting deep in the median furrow, it passes forward across the anterior surface of the forebrain; it then curves in the angle formed by the union of the olfactory tract and the forebrain, and finally passes along the inner margin of the tract to reach the median division of the *fila olfactoria*. It crosses this obliquely and enters the fissure between the two divisions of the *fila*.

For a review of the literature relating to the *nervus terminalis*, the paper by Larsell ('18) should be consulted.

To quote further from Locy ('99): "The olfactory nerve in selachians has for a long time been represented as double, but very little has been said about it in descriptions of figures or in texts." Locy ('99, '03) has described the development of the olfactorius in *Squalus acanthias*, showing that in its earlier stages it is distinctly double. Those who have figured the double character of the olfactory nerve in selachians have for the most part concerned themselves with the olfactory bulb. Miklucho-Maclay ('70), in many excellent figures of the brains of various selachians, has shown very plainly that the olfactory bulb is distinctly double. Locy draws a sharp distinction, as most of his predecessors have not done, between the olfactory *fila* (olfactory nerve) and the olfactory bulb and tract.

As Locy observes in the 150-mm. stage of *Squalus*, a distinction externally between bulb and tract can hardly be made. In section, however, there may be distinguished in the olfactory

lobe a posterior fibrous portion at whose periphery the olfactory glomeruli gradually appear, in passing anteriorly section by section. In the posterior part of the field of their appearance in the sections an anatomical distinction between lateral and ventral groupings of the glomeruli does not seem apparent, but proceed-

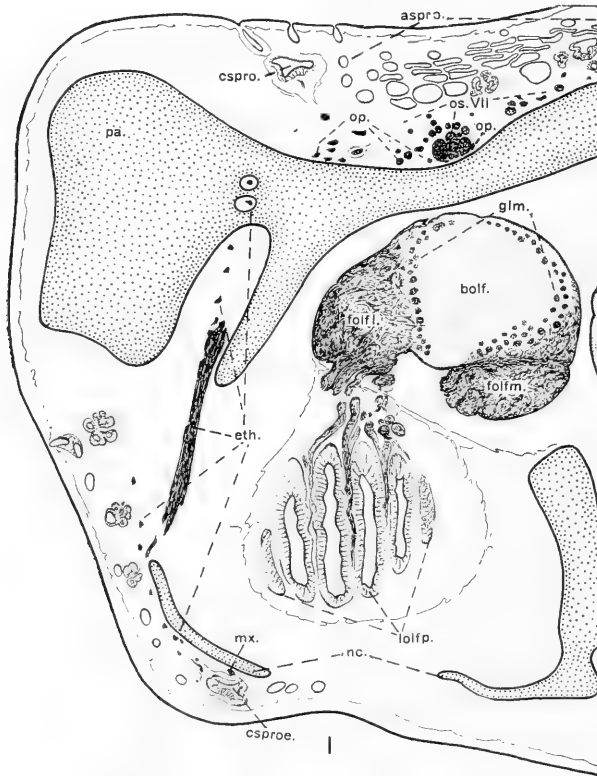


Fig. 1 A cross-section of the right half of the head through the olfactory bulb and the extreme posterior border of the olfactory lamellae. Section 429 (fig. 51). $\times 15$.

ing anteriorly there are soon seen three bands of glomeruli, dorsal, lateral, and ventral. Anterior to the level where the olfactory bulb appears separate from the brain in cross-sections, the dorsal and ventral areas unite mesially into one, ventromesial in position (fig. 1, *glm.*).

ABBREVIATIONS

- a.*, nerves supplying ampullae of Lorenzini
abas., basilar artery
abuc., buccal ampullae of Lorenzini
ac., acusticum (tuberculum acusticum)
admd., m. adductor mandibulae and its innervation
an., ala nasalis
aoph., ophthalmic artery
apsdbr., pseudobranchial artery
apstc., posterior cerebral artery
aspro., supraorbital ampullae of Lorenzini
auc., auditory capsule
ba., basal portion of cranium
bolf., bulbus olfactorius
bolf., lateral portion of the olfactory bulb
bolfm., mesial portion of the olfactory bulb
br.plx., brachial plexus
br.X1-4, branchial nerves of the vagus
buc., *buc.VII*, ramus buccalis VII
bv., unnamed blood-vessels
c., nerves supplying canal organs
care., external carotid artery
cari., internal carotid artery
cbi., cerebellum
cbr.1, first ceratobranchial cartilage
cd., chorda dorsalis
chm., hyomandibular lateral-line canal
chmd., hyomandibular cartilage
cil., ciliary nerves
cila., anterior ciliary nerve arising from the r. oph. prof. V
cilp., posterior ciliary nerve arising from the r. oph. prof. V
cilrl., radix longa of the ciliary plexus
cinfro., infraorbital lateral-line canal
clat., main lateral-line canal of the trunk
cmd., mandibular lateral-line canal
cplx., chorioid plexus
cr., cranial wall
crtf., corpus restiforme
csp., chorda spinalis
cspno., supraorbital lateral-line canal
csproe., ethmoidal section of the preceding
cspt., supratemporal lateral-line canal
ctm., temporal lateral-line canal
cv.1, first ventral constrictor muscle
dors.X, ramus dorsalis X
dlpo., dorsolateral series of pit-organs
dpo., dorsal series of pit-organs
eth., branch of r. oph. spf. VII innervating the ethmoidal section of the supraorbital lateral-line canal
flm., fasciculus longitudinalis medialis
folf., fila olfactoria
folfl., lateral division of the olfactory fila
folfm., mesial division of the olfactory fila
folfm.ad l., mesial olfactory fila distributed laterally
gac., ganglion acusticum
gacs., saccular portion of the auditory ganglion
gacv., vestibular portion of the auditory ganglion
gbr.X1-4, ganglia of the branchial nerves of the vagus
gbuc., ganglion of the ramus buccalis VII
gbuca., small accessory ganglion on the preceding
gcil.1-9, ganglia of the ciliary plexus
gg., ganglion Gasseri
ggen., ganglion geniculi
ggl., ganglion glossopharyngei
gll.X, lateral-line ganglia (in entirety) of the vagus nerve
gll.Xa, b, c, the three lateral-line ganglia of the vagus nerve
glm., glomeruli olfactorii
gmde., ganglion of the ramus mandibularis externus VII
gop., ganglion of the ramus oph. profundus V

- gos.*, *gos.VII*, ganglion of the ramus oph. superficialis VII
gos.V, ganglion of the ramus oph. superficialis V
got., ganglion of the ramus oticus VII
gspt.IX, lateral-line ganglion of the glossopharyngeal nerve
gsy., sympathetic ganglia
gv., ganglion vagi
g.IX, combined ganglia of the glossopharyngeal nerve
hybr., nervus hypobranchialis
hy., *hy.VII*, ramus hyoideus VII
ical.1, first intercalary cartilage
lat.X, ramus lateralis X
lbinf., lobi inferiores
lbl., lobus lineae lateralis
lbvs., lobus visceralis
lolf., anterior olfactory lamellae
lolfp., posterior olfactory lamellae
lvp-q., m. levator palato-quadrati and its innervation, including that of the m. spiracularis
md., *md.V*, ramus mandibularis V
mde., *mde.VII*, ramus mandibularis externus VII
mdea., *mdep.*, anterior and posterior divisions of the r. mde. VII
mdi., *mdi.VII*, ramus mandibularis internus VII
mdobl., medulla oblongata
mdpo., mandibular series of pit-organs and their innervation
mes., mesencephalon
mes.V, radix mesencephalica V
mx., *mx.V*, ramus maxillaris V
mxpc., a posterior cutaneous branch of the ramus maxillaris V
mxph., pharyngeal branch of the ramus maxillaris V
nas.d., anterior dorsal division of the nasal chamber
nas.v., posterior ventral division of the nasal chamber
nc., nasal cartilage and capsule
nflv., nasal flap valve
nt., nervus terminalis
oc., eyeball
occ., occipital condyle
occ. y+z, occipital nerves y and z
oi., m. obliquus inferior and its innervation
op., *op.V*, ramus ophthalmicus profundus V
opch., optic chiasma
os.V, ramus ophthalmicus superficialis V
os.V1-4, branches of the preceding
os.VII, ramus ophthalmicus superficialis VII
ospr., spiracular sense organ
ot., *ot.VII*, ramus oticus VII
pa., processus antorbitalis
pal., *pal.VII*, ramus palatinus VII
pch., parachordal region of cranium
ped., optic pedicel
ph.IX, *ph.X1-4*, pharyngeal rami of the glossopharyngeal and vagus nerves
pmn.V, portio minor and its rootlets of the trigeminal nerve roots
pmj.V, portio major of the trigeminal nerve roots
p-q., palatoquadrate cartilage
pro., m. preorbitalis and its innervation
prt.VII, *prt.IX*, *prt.X1-4*, pretrematic rami of the facial, glossopharyngeal and vagus nerves
pst.IX, *pst.X1-4*, posttrematic rami of the glossopharyngeal and vagus nerves
pst.IXa, *pst.Xa1-4*, accessory posttrematic rami of the glossopharyngeal and vagus nerves
r., rostrum
rbuc., root fibers of the ramus buccalis VII
rc., rostral carina
rect., m. rectus lateralis (externus) and its innervation
rinf., m. rectus ventralis (inferior) and its innervation
rint., m. rectus internus and its innervation
rot., root fibers of the ramus oticus VII

rs., m. rectus dorsalis (superior) and its innervation
sac., sacculus
sacvs., saccus vasculosus
sbsp., m. subspinalis and its innervation
sca., anterior semicircular canal
scl., sclerotic coat of the eyeball
so., m. obliquus dorsalis (superior) and its innervation
sp.1-6, first six spinal nerves
sp.1m., motor portion of the first spinal nerve
sp.1rm., motor root of the first spinal nerve
sp.1s., sensory portion of the first spinal nerve
sp.3m., motor portion of the third spinal nerve
sp.3s., sensory portion of the third spinal nerve
spr., spiracle
sptpo., supratemporal pit-organs
spt.IX, ramus supratemporalis IX
spt.X, ramus supratemporalis X
sp.V, tractus spinalis trigemini
sp.Va, anterior continuation of the preceding
sy., sympathetic nerves
tm., tectum mesencephali
trap., m. trapezius and its innervation
tr.hmd.VII, truncus hyomandibularis VII
tr.io.V+VII, truncus infraorbitalis (mx.V+buc.VII)
trm., trunk muscles
utrc., utriculus
v, w, x, y, z, the five occipital nerves
v.1, first vertebra
visc.X, ramus visceralis X
vpo., ventral series of pit-organs and their innervation

I, nervus olfactorius
II, nervus opticus
III, nervus oculomotorius
IIId., dorsal division of the oculomotor nerve
IIIv., ventral division of the oculomotor nerve
IV, nervus trochlearis
IVch., decussation of the trochlear nerve
IVndl., nidulus of origin of the trochlear nerve
IVr., root of the trochlear nerve
Vrm., motor root of the trigeminal nerve
VI, nervus abducens
VII, nervus facialis
VIIrll., lateral-line roots of the facial nerve
VIIrllld., dorsal lateral-line root of the facial nerve
VIIrllv., ventral lateral-line root of the facial nerve
VIIrm., motor root of the facial nerve
VIIrvs., visceral sensory root of the facial nerve
VIII, auditory nerve
VIIIr., root of the auditory nerve
VIIIIs., saccular division of the auditory nerve
VIIIv., vestibular division of the auditory nerve
IXr., root of the glossopharyngeal nerve
Xr., roots of the vagus nerve proper
Xr.br.1-4, roots of the four branchial nerves of the vagus
Xrll., lateral-line roots of the vagus
Xrm., motor rootlets of the vagus
Xrvs., visceral sensory rootlets of the vagus

Locy has stated that the olfactory cup in selachians is imperfectly divided by the development of a membranous fold from the ring surrounding the entrance to the cavity, there resulting two parts in the chamber, a mesial and a lateral. These two

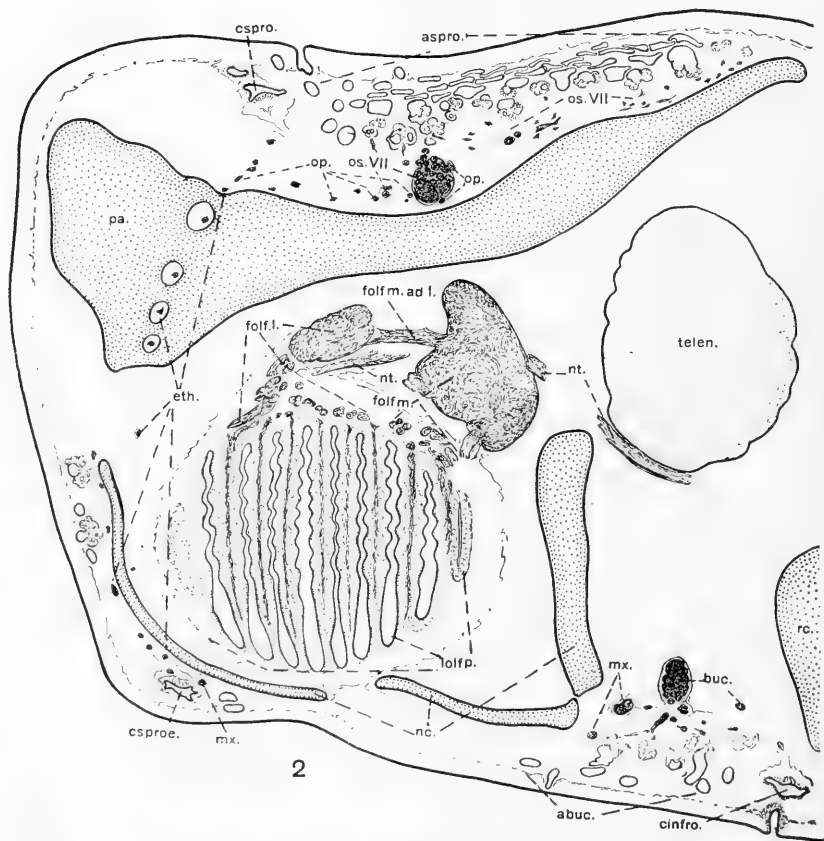


Fig. 2 A similar section slightly anterior to the preceding, and immediately anterior to the olfactory bulb, through the two masses of olfactory fila. Section 412. $\times 15$.

divisions in the olfactory cup are the result, as Berliner ('02) has shown, of the early differentiation in the embryo of two groups of Schneiderian folds or lamellae. These, however, have no exact relation to the double nature of the olfactory nerve. Sund

('04, '05) finds in *Spinax niger* that the olfactory nerve in its development is at first single, connecting the anterior ventral part of the olfactory placode with the brain. Later by shifting and extension of the olfactory pit the nerve becomes connected with the dorsal part of the placode. Longitudinal Schneiderian

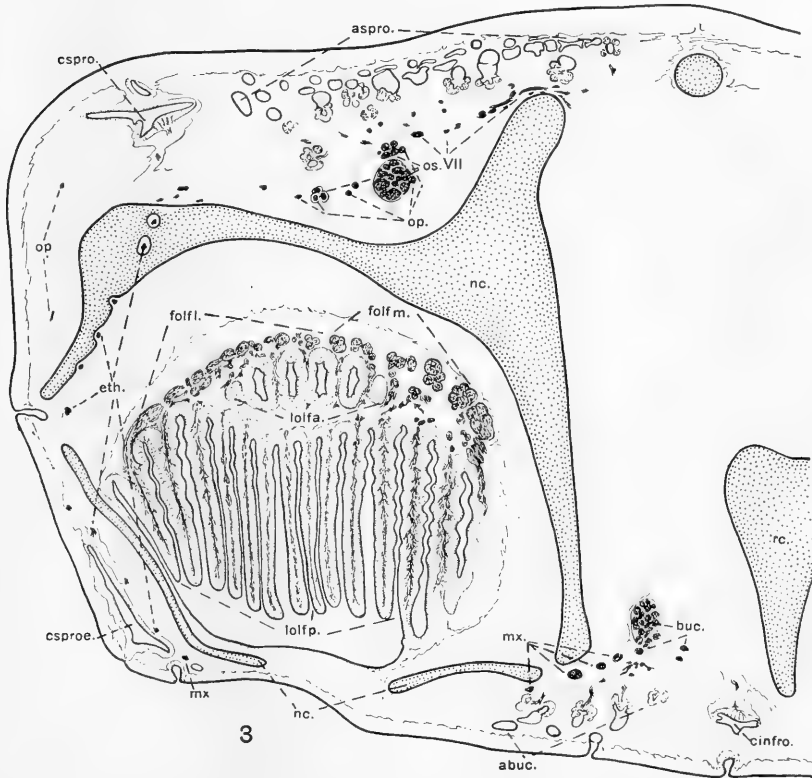


Fig. 3 A similar section through the nasal capsule, cutting the extreme posterior edge of the lamellae of the anterodorsal part of the olfactory cup. Section 387. $\times 15$.

folds appear, most complete near the nerve. Later a secondary group of the folds appears and a secondary nerve connection with the anteroventral part of the placode occurs. There results a double chamber—one posterior, containing the primary folds and related to the primary nerve, the other anterior, containing the

secondary folds and related to the secondary nerve. Sund suggests that the anterior secondary chamber corresponds to Jacobson's organ. Asai ('13) shows in models of the olfactory organ of *Mustelus laevis* that the olfactory cup is divided by the membranous fold into a posteroventral and an anterodorsal chamber,

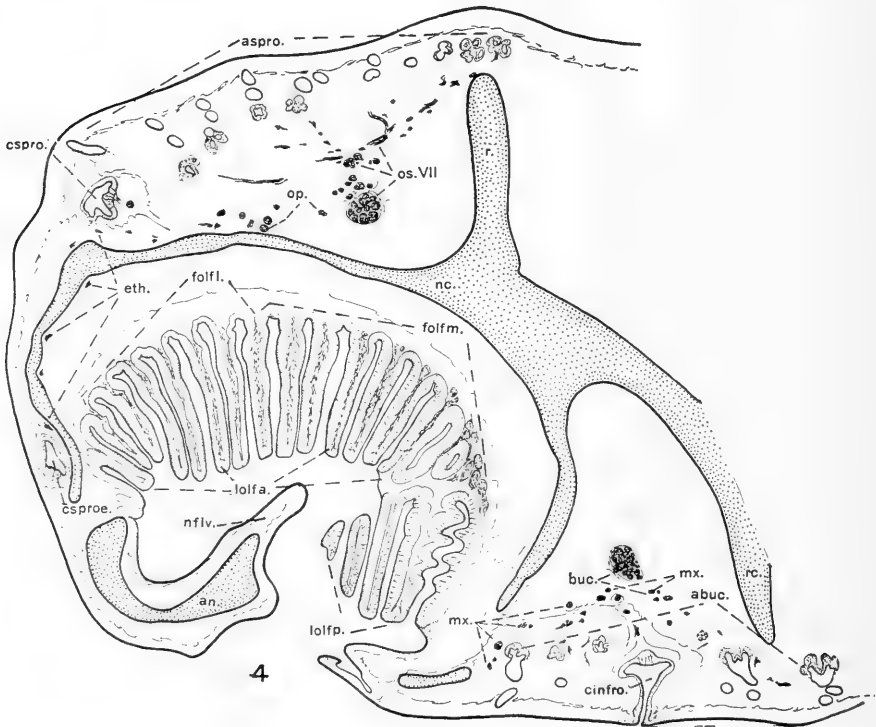


Fig. 4 A cross-section through the right olfactory cup, cutting through the nasal opening and the extreme anterior edge of the lamellae of the posteroventral part of the olfactory chamber. Section 346. $\times 15$.

but that the two series of Schneiderian folds are continuous with each other around the ends of the septum.

In *Squalus acanthias* in the 150-mm. stage these two parts of the olfactory cup are posteroventral and anterodorsal in position. The olfactory fila entering the mesial (dorsal, mesial, ventral) group of glomeruli in the olfactory bulb are related to the larger

part, mesial and lateral of the olfactory lamellae in the anterodorsal portion of the olfactory cup, and to a smaller mesial portion of the lamellae in the posteroventral part. The fila entering the lateral group of glomeruli are from the larger lateral part of the lamellae of the posteroventral portion of the cup, and from a smaller number of the lateral lamellae of the anterodorsal part of the cup. In brief: the lateral olfactory glomeruli are related to the lateral olfactory lamellae in the olfactory cup; the mesial glomeruli are connected with mesial lamellae and with some of the lateral ones. Thus there are two olfactory nerves: a ventromesial olfactory nerve, related on the one hand to dorsal, mesial, and ventral glomeruli, and on the other to mesial and lateral lamellae in both parts of the olfactory cup; and a lateral olfactory nerve, related to lateral glomeruli and to lateral lamellae in both parts of the cup. Hence the two divisions of the olfactory cup are not in exact correspondence to the two olfactory nerves, as Locy supposes. The lateral division of the olfactory fila evidently corresponds to Sund's primary olfactory nerve, and the mesial division to the secondary nerve, but later distributions of fibers have obscured the original sharp distinction between the two nerves. In *Mustelus* the relations of the olfactory fila to the olfactory cup and parts of the olfactory bulb are essentially as in *Squalus*.

In figure 1 is shown a cross-section through the anterior portion of the olfactory bulb, where the olfactory glomeruli are seen arranged in two groups: a dorsal-mesial-ventral series and a lateral series. The two large masses of olfactory fila, ventromesial and lateral, give a distinctly double appearance to the anterior part of the bulb (figs. 35, 51, 52, 53, *bolfm.*, *bolfl.*). Figure 2 is of a cross-section through the two olfactory nerves slightly posterior to the transverse level where the *nervus terminalis* passes across the mesial olfactory fila. Some of the lateral olfactory fila are seen passing mesially into the mass of mesial fila (*folfm. ad l.*). In figure 3 the posterior edge of the anterodorsal olfactory lamellae is shown, and in figure 4 the anterior extremities of the posteroventral lamellae. The position of the septum between the two parts of the olfactory cup is indicated in figures 35 and 51.

THE OPTIC AND THE EYE-MUSCLE NERVES

The thoroughgoing descriptions and discussions by Neal ('98, '14, '18) of the origin, histogenesis, homologies, and general topographical relations of the eye-muscle nerves, with especial relation to the condition in *Squalus acanthias*, make any extended treatment of the subject by the present writers quite superfluous. For a review and list of the literature upon this subject the reader is referred to the second ('14) of these papers by Neal.

From the eyeball the optic nerve runs posteromesially into the optic foramen, and thence anteriorly into the chiasma. The anterior dorsal part of the chiasma is formed from fibers situated dorsally in the nerve, and the ventral posterior part from the ventral portion of the nerve. Near the brain a cross-section of the nerve shows a deep notch on its posterior border; near the eyeball a similar section reveals four such deep indentations (figs. 15 and 34).

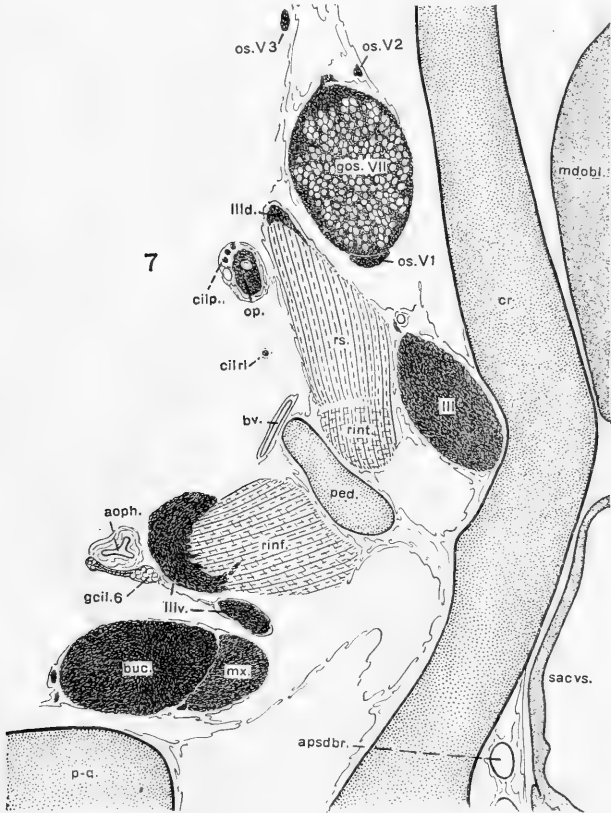
The nidulus of origin of the oculomotor nerve is situated immediately dorsal to the ventral motor columns, slightly posterior to a transverse level where the cavity of the midbrain communicates ventrally with that of the inferior lobes. Numerous strands of fibers, many of which decussate, pass posteroventrally from the nidulus, emerging immediately posterior to the point where in cross-section the inferior lobes separate externally from the midbrain. After being collected into the external nerve, the rootlets pass posteriorly in the angle between the midbrain and the inferior lobes, the nerve lying at first at the dorsolateral angle of the inferior lobes, farther posteriorly in the same relation to the saccus vasculosus, finally passing laterally across the dorsal border of the latter to reach its exit from the skull (figs. 5, 6, 12 to 14, and 21). An intracranial ganglion on the oculomotorius, as reported by Nicholls ('15) in *Scyllium*, is not found by the writers in *Squalus*. Entering the orbit the third nerve, turning sharply anteriorly, almost immediately divides into two branches. An anterior dorsal division (*IIId.*) passes at once into contact with the dorsal rectus muscle, and running along its anteromesial border between it and the internal rectus muscle



Figs. 5 to 11 Parts of cross-sections through the orbit of the right eye. To show the interrelations of the oculomotorius, the trigeminal and facial trunks in the orbit, the ciliary plexus and the ocular muscles. $\times 25$. Fig. 5, section 859. Fig. 6, section 869. Fig. 7, section 889. Fig. 8, section 893. Fig. 9, section 895. Fig. 10, section 901. Fig. 11, section 906.

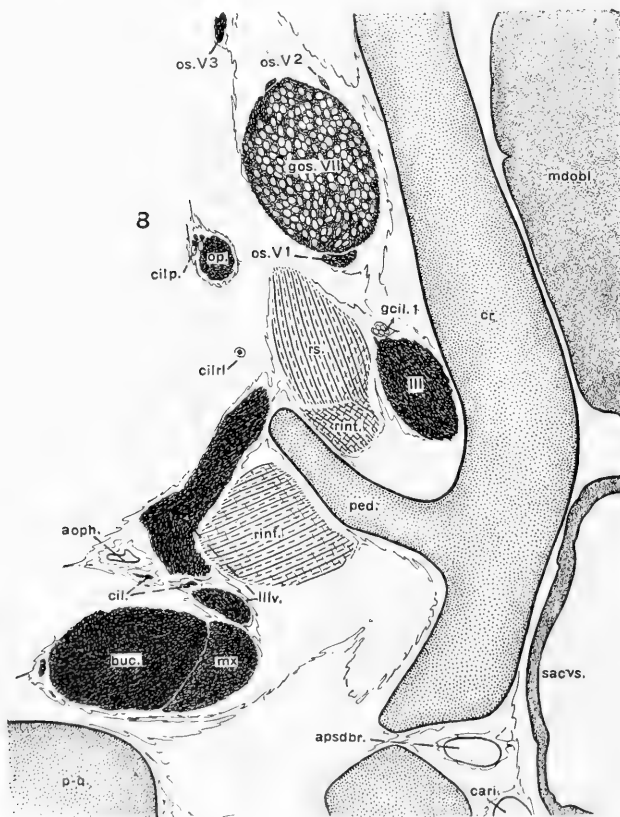


innervates both muscles (figs. 5 to 7, 15 to 17, and 21). The posterior ventral division of the nerve turns posteriorly and then sharply ventrally around the origins of the rectus dorsalis and rectus internus on the optic stalk, passing between the ganglion of the ramus ophthalmicus profundus and the rectus dorsalis



muscle (fig. 31, *IIIv.*), then between the optic stalk and the rectus lateralis muscle, farther ventrally between the latter and the origin of the rectus internus, and still farther ventrally between the rectus lateralis and the rectus ventralis (figs. 8 to 11, 15 to 17, 20, and 21). In thus passing ventrally it divides into an anterior and a posterior branch, both divisions turning

anteriorly around the posterior border of the rectus ventralis, the anterior one entering and innervating the muscle and the posterior one running across the ventral border of the muscle and parallel to the truncus infra-orbitalis (figs. 7 to 9, 15 to 17, and 21). This relation to the latter nerve is maintained until



the oculomotor branch terminates in the obliquus ventralis muscle (figs. 5 to 9, 15 to 17, and 21). In this anterior course the nerve passes ventral to the optic nerve. The relations of the oculomotor nerve to the ciliary plexus will be described later.

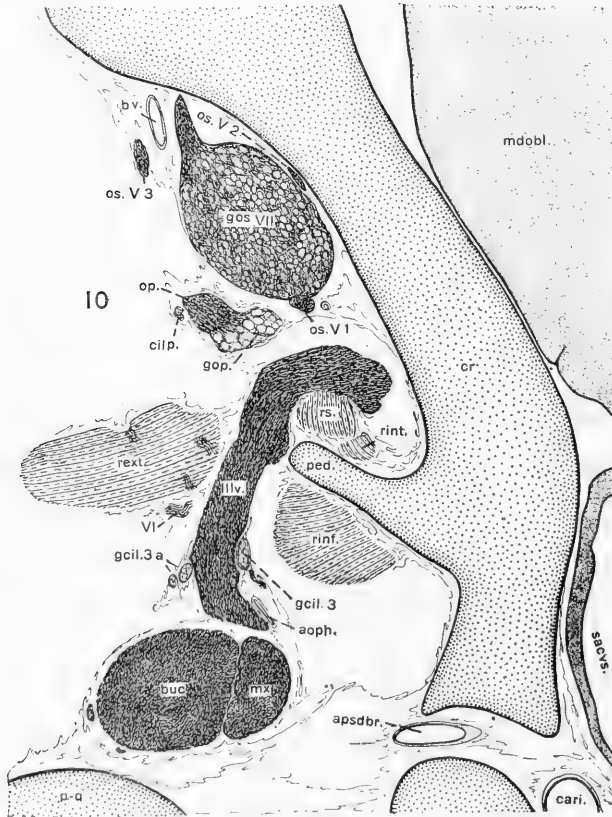
The trochlear nerve leaves the brain in the fashion characteristic of selachians: on the dorsal wall of the midbrain just an-

terior to the cerebellar stalk, and ventral to the cerebellar crest (figs. 18 and 24). At its decussation in the velum it becomes closely associated with other fiber tracts: the decussatio veli, the tractus tectocerebellaris, and the radix mesencephalica trigemini. The relations of these various tracts to each other have



been described by Johnston ('05, '09) and by van Valkenburg ('11) in *Scyllium canicula* and *stellare*. As stated by these authors, the relations in *Squalus acanthias* (*Acanthias vulgaris*) are essentially similar to those in *Scyllium*. From the decussation the trochlearis fibers pass anteroventrally around the lateral angle of the fourth ventricle to their origin in the troch-

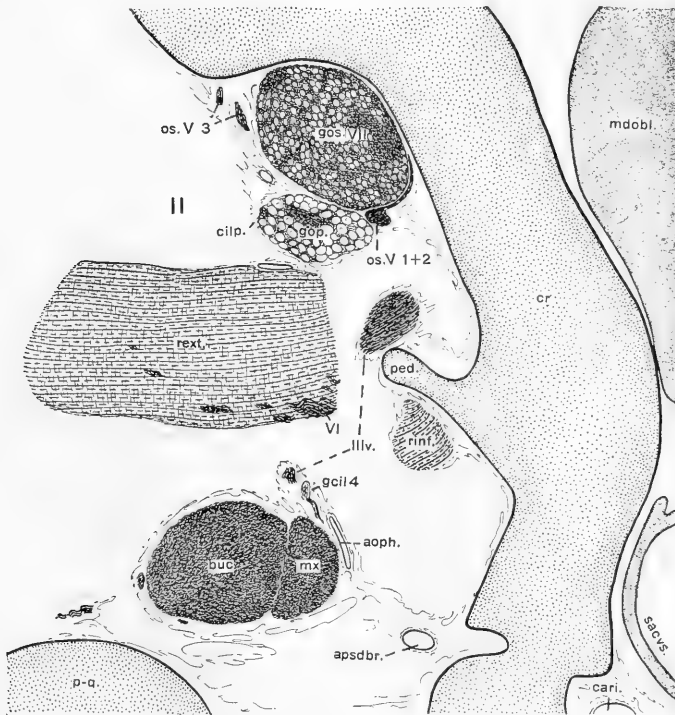
lear nidulus dorsal to the ventral motor fiber columns immediately posterior to the oculomotor nidulus (figs. 12 to 14, 18, and 19). Near the decussation the trochlear fibers pass by and interlace with the fibers of the radix mesencephalica V, which here consists of numerous small tracts near their termination in the mesen-



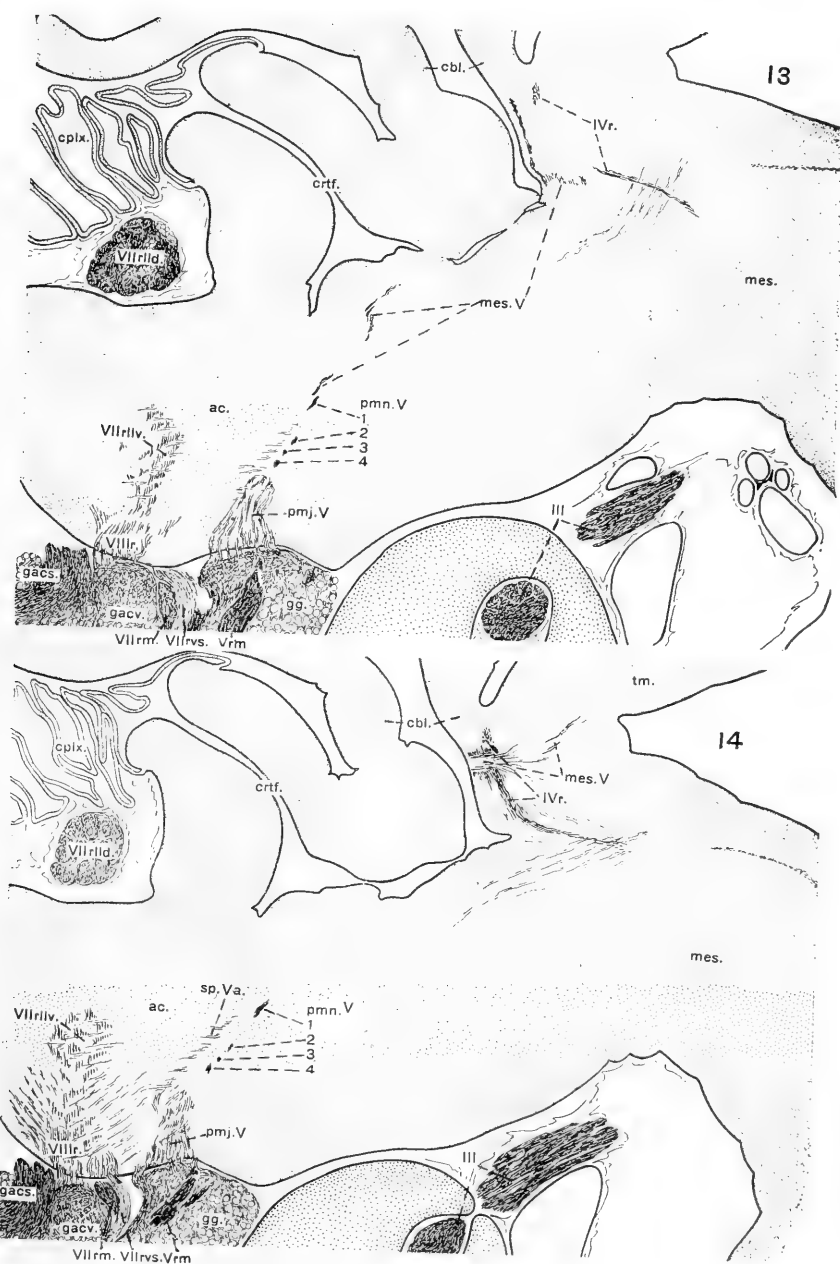
cephalic tectum. Peripherally, the nerve runs anteriorly along the dorsolateral border of the midbrain, in the anterior part of its intracranial course pressed closely between the brain and the skull. At its foramen it passes ventrolaterally through the cranial wall and comes into intimate relation with the mesial border of the ramus ophthalmicus superficialis facialis. Thence

passing anteroventrally around the ventral border of the latter, it turns abruptly anterodorsally across the orbital cavity and soon reaches the dorsal oblique muscle which it innervates.

The posterior rootlets of the abducens nerve appear emerging from the ventral motor column of the medulla oblongata at the transverse level of the anterior border of the internal opening of the spiracle (fig. 20). There are about eight large rootlets that



contribute to its formation, all arising in the ventral motor column. Peripherally, the nerve runs anteriorly intracranially, at first at the ventrolateral border of the medulla; farther anteriorly at the transverse level of the posterior border of the gasserian ganglion, it enters a canal in the ventral floor of the cranium and runs anterolaterally toward the orbit. Emerging from the canal mesial to the maxillary ramus of the trigeminal nerve, it passes



around the anterior border of this nerve, turns laterally into the orbit, and divides into two branches, one of which immediately enters the anterodorsal part of the rectus lateralis muscle, the other the anteroventral part (figs. 10, 11, 15 to 17, and 20).

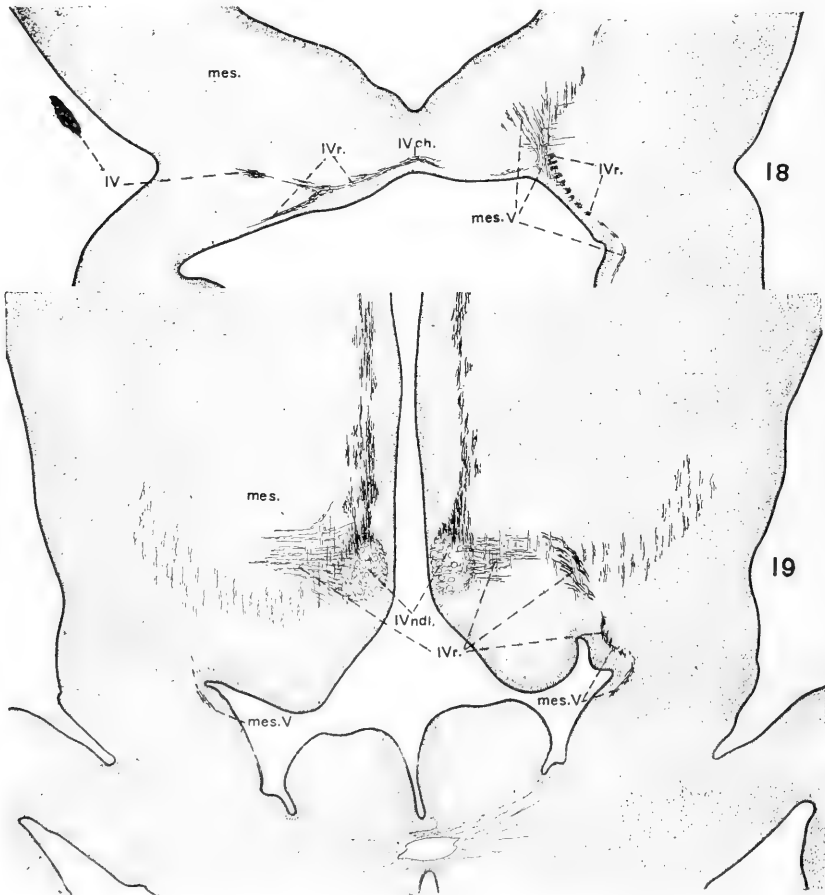


Fig. 18 A horizontal section through the trochlear decussation, showing the relations of the trochlear roots and the radix mesencephalica V. Section cut somewhat obliquely. $\times 25$.

Fig. 19 A horizontal section through the trochlear nidulus, showing the relations of the trochlear root and the radix mesencephalica V. $\times 25$.

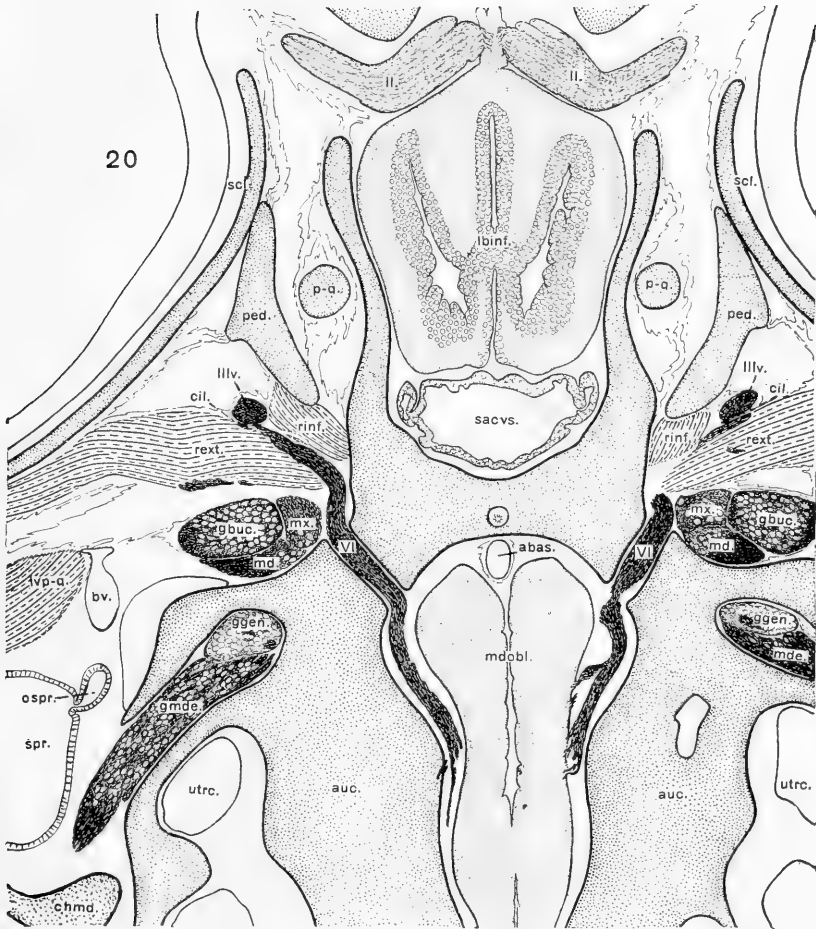


Fig. 20 A horizontal section through a portion of the base of the cranium from the optic commissure anteriorly to the anterior portion of the ear capsule posteriorly, cutting the optic chiasma, the roots of the abducens nerve, the inferior lobes, the saccus vasculosus and the anterior part of the medulla oblongata. $\times 15$.

THE TRIGEMINAL NERVE

1. *The roots and ganglia of the trigeminal complex*

According to Landacre ('16), the roots of the trigeminal nerve in 22-mm. embryos of *Squalus acanthias* are in two groups: (I) an anterior portio minor of three roots: 1) sensory fibers by which the profundus ganglion connects with the tractus spinalis trigemini; 2) motor fibers; 3) sensory fibers, presumably belonging to the maxillomandibular division of the fifth nerve; (II) a posterior portio major, consisting of sensory fibers entering the spinal V tract. At the stage examined by the writers there are found in the portio minor four or five rootlets: 1) an anterior dorsal rootlet of motor fibers, with which are possibly associated sensory elements; 2) motor fibers; 3) fibers ending in the spinal V tract, and presumably sensory; 4 and 5) motor fibers (figs. 12 to 14, 21, 22, and 25 to 30, *pmn.V*, 1-5). The portio major consists of a large number of sensory rootlets entering the spinal V tract, with which are mingled a small number of motor fibers (figs. 12 to 14, 21, 22, 29, 30, and 32, *pmj.V*).

That the first rootlet of the portio minor is largely motor is beyond question, but its exact composition the writers have found well nigh impossible to determine. That fibers from the profundus ganglion enter the brain through this first rootlet seems possible; they appear to do so. In fact, the relations in the gasserian ganglion would justify the supposition that all the rootlets of the portio minor contain sensory elements, but their course in the brain does not warrant such a conclusion. The first rootlet passes directly into the anterior continuation of the spinal V tract, where it divides into two parts, one continuing on directly into the lateral motor column, the other turning abruptly anteriorly and becoming the radix mesencephalica V. Appearances permit the assumption that some fibers of this first rootlet may end in the spinal V tract. The second rootlet seems to be exclusively motor. It passes through the spinal V tract, but shows no indications of giving fibers to it. The third rootlet is very small. It arises immediately posterior to the second rootlet, and in some specimens cannot be recognized, probably being

united with the preceding rootlet. It ends in the spinal V tract. It seems to come from the profundus ganglion rather than from the gasserian. The fourth and fifth rootlets may possibly con-

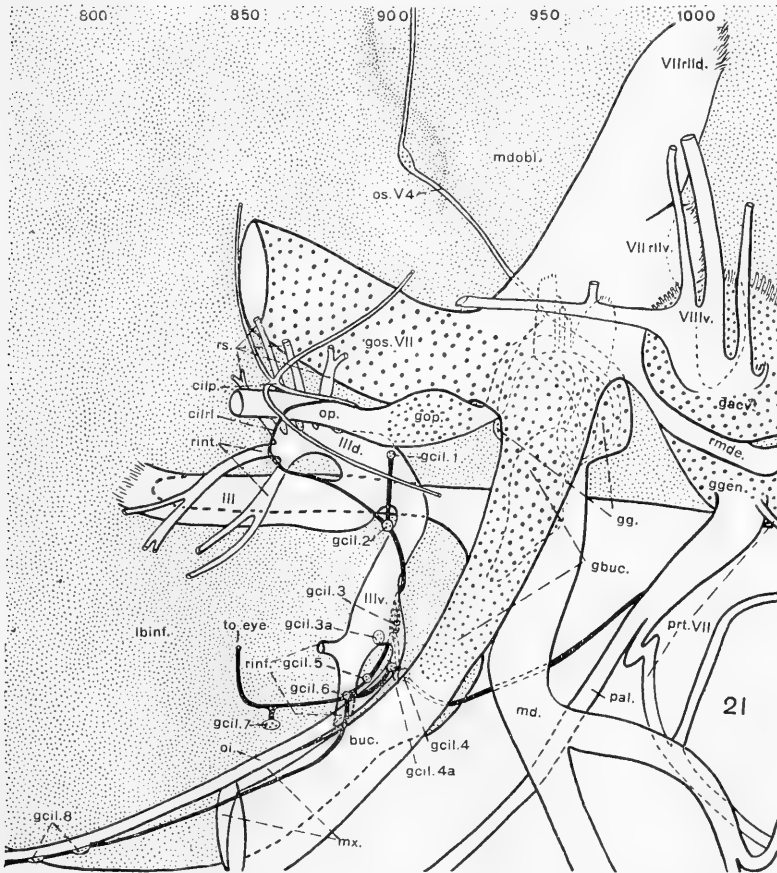


Fig. 21 A projection upon the sagittal plane of the roots and ganglia of the V-VII-VIII nerve complex, together with the oculomotor nerve and the ciliary sympathetic nerve plexus. Represented as seen from the left side. $\times 20$.

tain sensory fibers, but motor elements predominate. From a study of cross, sagittal, and horizontal sections the impression is gained that the portio minor is overwhelmingly motor in composition, and that the greater part of the profundus fibers enter

the portio major. The latter contains only a very small percentage of the motor elements in the trigeminal nerve. The sensory fibers of the portio major on entering the brain pass by

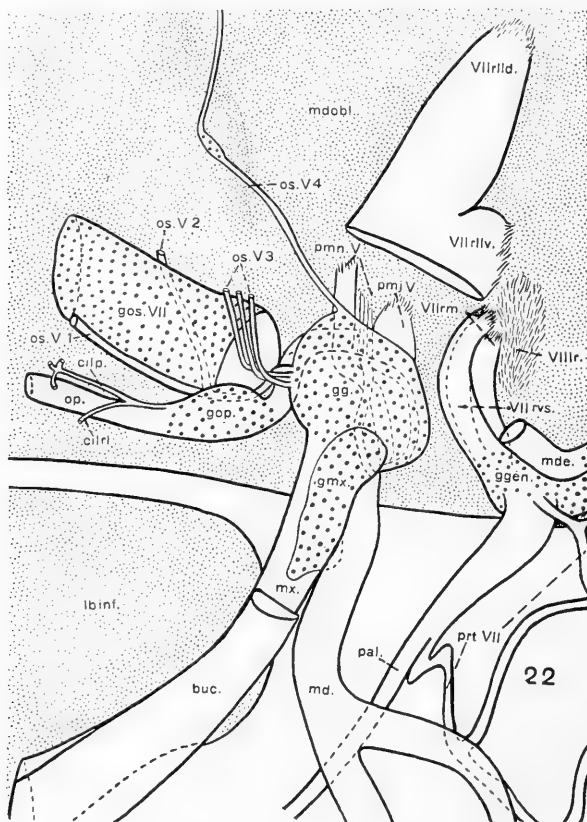


Fig. 22 A projection upon the sagittal plane of the roots and ganglia of the V-VII-VIII nerve complex, portions of the lateral-line roots and ganglia being represented as cut away to expose the gasserian ganglion with its roots and the roots of the facialis proper. Of the auditory nerve only the root is shown. Left view. $\times 20$.

a broad sweeping curve into the tractus spinalis trigemini, running ventral to the motor and visceral sensory roots of the facialis. The condition in *Squalus* gives no justification for the statement that "the fibers of the ophthalmicus profundus are

traced into the midbrain." The relations of the roots of the fifth nerve in *Mustelus* are essentially as in *Squalus*.

Marshall and Spencer ('81), summarizing the studies of other investigators, state for the trigeminal nerve in adult selachians

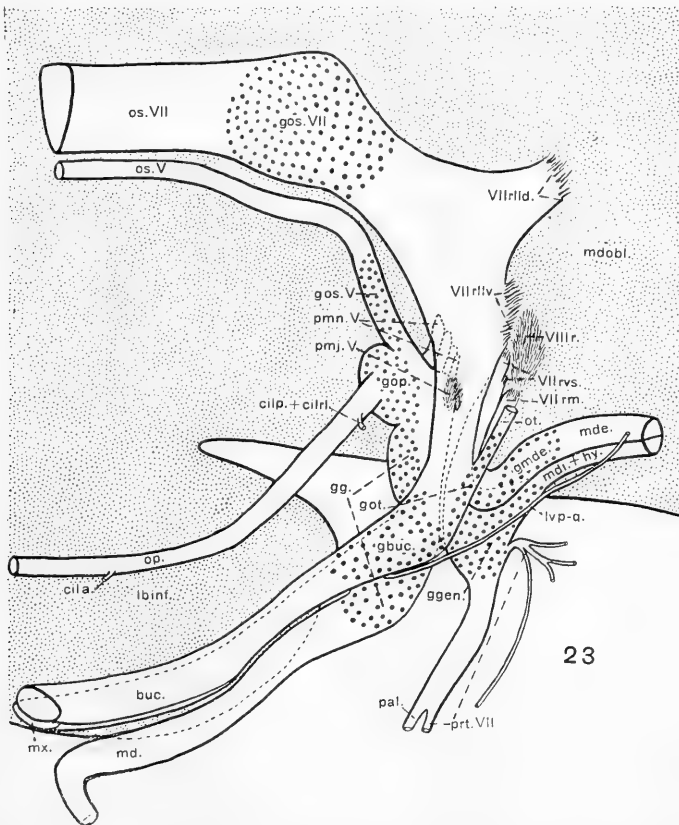
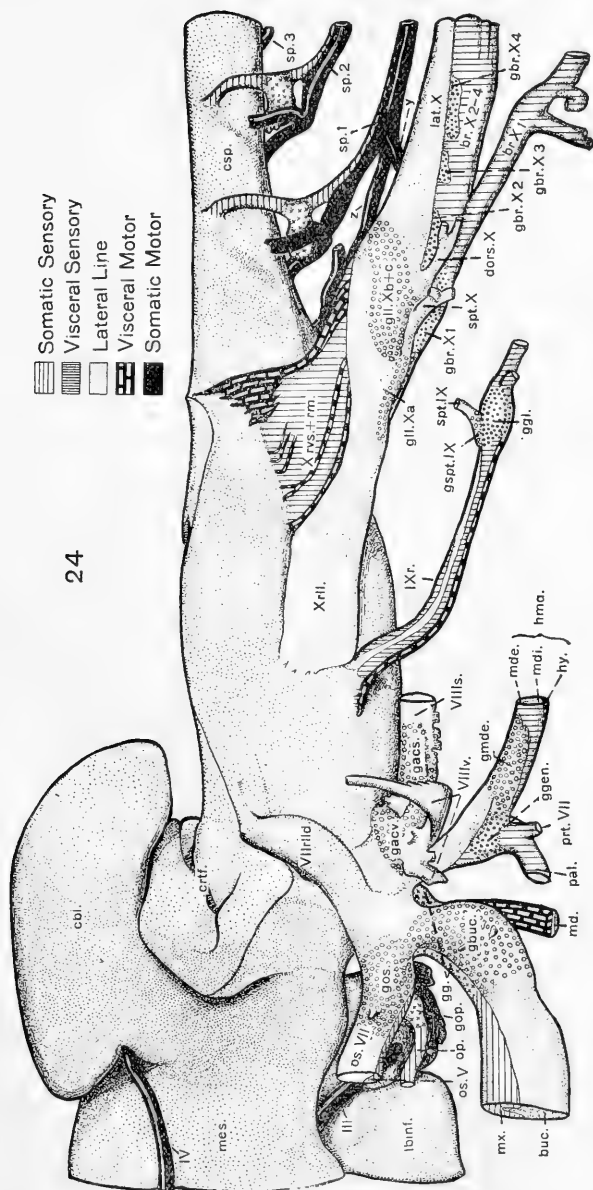


Fig. 23 A projection upon the sagittal plane of the roots and ganglia of the V-VII-VIII nerve complex of *Mustelus californicus*. Left view. $\times 20$.

two roots: 1) an anterior one [portio minor] arising from the brain by two non-ganglionated rootlets; 2) a posterior ganglionated root [portio major]. Van Valkenburg's more recent description of the roots of the trigeminus in *Scyllium* ('11 a and b) indicates that the roots of the fifth nerve in that form are essentially

as in *Squalus*. Van Wijhe ('82) finds in stage L of the embryo of *Scyllium* that the trigeminal nerve connects with the brain by two roots, an anterior non-ganglionated part, which he regards as belonging to the ramus ophthalmicus profundus, and a posterior ganglionated root. Mitrophanow ('92) antagonizes the views of van Wijhe, and while controverting the opinion that the ophthalmicus profundus is an independent nerve asserts that in *Acanthias vulgaris* the anterior trigeminal root [portio minor] belongs with the maxillomandibular trunk, the ophthalmicus profundus sending its fibers chiefly into the posterior root [portio major]. Ewart ('89) states that in *Laemargus* the ramus ophthalmicus profundus arises by two to five rootlets immediately in front of the main trigeminal root; but this is doubtless an error, for the probability is that the relations that obtain in *Squalus*, *Scyllium*, and *Mustelus* are typical. The discrepancy between this account of the roots of the trigeminus in *Squalus* and the description by Landacre is doubtless to be explained as due largely to a later and more extensive development of motor fibers in the stages studied by the writers.

The ganglia of the two divisions of the trigeminal nerve are sharply distinct in *Squalus*, both in embryo and adult. The profundus ganglion (*gop.*), wholly extracranial in position, is in contact dorsally with the anterior (dorsal) lateral-line ganglion, which sweeps out in a semicircle laterally, nearly hiding the trigeminal ganglia (figs. 10, 11; 16, 17, 21, 22, 24, 31, 32, 35, 51, and 52). The profundus ganglion in the 'pup' stage is about 500μ long and oval in shape. Its root fibers pass posteriorly at the lateroventral border of the anterior portion of the lateral-line ganglion, accompanied on its mesial border by fibers of the ramus ophthalmicus superficialis trigemini (figs. 10, 11, 22, and 35, *os.V1*). It is difficult to distinguish sharply between the two nerves in the common mass which they form as they approach the brain, but as they pass toward the brain wall the profundus fibers shift from a ventrolateral to a dorsomesial position (fig. 22). Reaching the anterior dorsal tip of the gasserian ganglion, the profundus fibers pass through to enter largely if not wholly the portio major, as described above, while the fibers



of the ramus ophthalmicus superficialis V enter the gasserian ganglion to become ganglionated. The further destination of the latter fibers is uncertain.

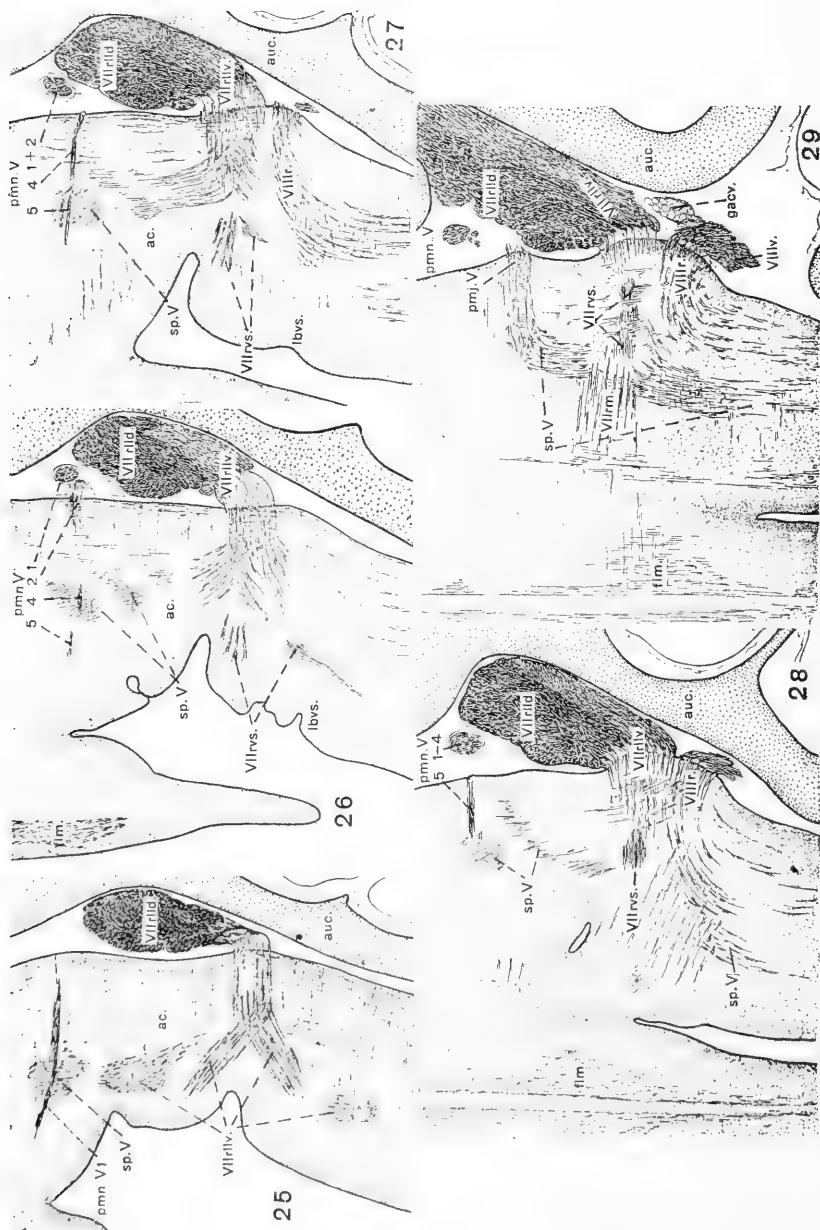
The gasserian ganglion is partly extracranial, extending out ventrolaterally from the brain wall, and in consequence is dumb-bell or hour-glass in shape, the intracranial part being somewhat larger (fig. 33, *gg.*, *gmx.*). This peculiar shape of the ganglion is due to the proximity of the lateral-line ganglion and the rectus lateralis muscle. From the proximal intracranial portion the mandibular and superficial ophthalmic rami arise, and from the distal portion the maxillary ramus (figs. 21, 22, and 24). Whether any of the sensory fibers of the portio minor belong to the gasserian ganglion is uncertain, but Landacre believes those of the third rootlet do. The gasserian ganglion is in contact anteriorly with the base of the profundus ganglion and the rectus lateralis muscle (figs. 16, 31, and 33), dorsally with the lateral-line roots of the facialis nerve (figs. 16 and 24), laterally with the dorsal lateral-line ganglion and root (fig. 16), and dorsolaterally with the dorsal lateral-line ganglion. Unlike the condition in the 22-mm. embryo, as described by Landacre, the emergence of the fibers of the ophthalmicus superficialis V does modify the form of the gasserian ganglion. Distal projections of small ganglionic masses are seen to be related to parts of the ramus ophthalmicus superficialis V (figs. 16 and 31).

In *Mustelus* the fifth nerve possesses three ganglia, merged posteriorly, as Allis finds, but nevertheless distinct: a dorsal ganglion of the r. oph. spf. V, a lateral profundus ganglion, and a ventral maxillomandibular ganglion. The first two of these are intracranial; the maxillomandibular ganglion is largely intracranial, but a considerable portion, as in *Squalus*, extends out through the trigeminal-facial foramen into the ventral portion of the orbit. Figure 23 is a projection upon the sagittal plane of the ganglia of the V-VII-VIII complex in *Mustelus californicus*, showing the relative independence of the several ganglia. At no place is there any difficulty in distinguishing between the trigeminal and facial ganglionic elements.

2. *The radix mesencephalica V*

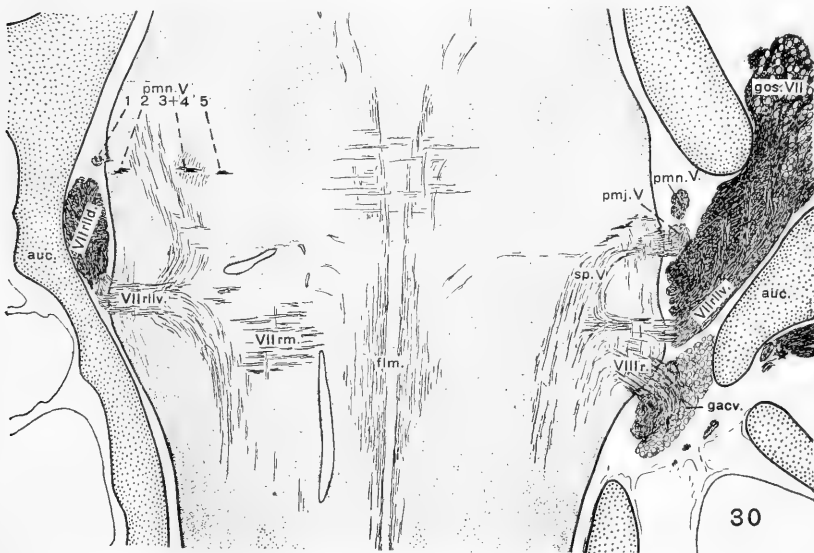
The radix mesencephalica V is formed in *Squalus* from the anterior rootlet of the portio minor of the trigeminus roots. This anterior rootlet passes in through the brain substance almost exactly at a right angle to the longitudinal axis of the medulla (fig. 25, *pmn.V1*). In it can be distinguished two constituents, a dorsal and a ventral (fig. 13, *pmn.V1, mes.V*). On reaching the anterior continuation of the tractus spinalis trigemini, the dorsal fibers turn abruptly within the latter as the radix mesencephalica V, and the ventral fibers pass directly mesially into the lateral motor column. The mes. V continues anteriorly and dorsally, ventrolateral to the lateral angle of the fourth ventricle. Before the posterior peduncle of the cerebellum is reached, the fibers of the mes. V have separated from those of the spinal V tract, and curving around the lateral angle of the ventricle ascend rapidly in the base of the cerebellum, in their passage losing their compact arrangement and taking the form of a diffuse fibrous band as they pass along the lateral wall of the ventricle (fig. 12). Reaching the transverse level of the posterior border of the mid-brain, the radix curves around the lateral border of a fissure-like lateral extension of the fourth ventricle in the lateral wall of the cerebellar segment (fig. 19), dividing into two limbs, of which a lateral one sends out branches into the lateral substance of the cerebellum. The mesial limb of the radix meets the root of the trochlearis rising from its nidulus situated anteriorly and ventrally (figs. 12 to 14, 18 and 19). The two tracts pass through each other, interlacing in a manner difficult of analysis, the trochlearis passing posteriorly and dorsally from the place of crossing to its decussation in the posterodorsal wall of the mid-brain. The radix after passing through the trochlearis divides diffusely and is distributed to the mesencephalic tectum, presumably to the nidulus magnocellaris (figs. 14 and 18).

According to Neal and others, the radix mesencephalica V in *Squalus* is a motor tract originating in the nidulus magnocellaris and passing out of the brain peripherally through the maxillo-mandibular division of the trigeminus. The writers agree with



Figs. 25 to 30 A series of horizontal sections through the roots of the V-VII-VIII nerve complex of the right side, beginning dorsally with a section through the ventral lateral-line root of the facial nerve and the first rootlet of the portio minor of the trigeminal nerve, and ending ventrally with a section through the root of the auditory nerve and the portio major of the trigeminal. As may be seen in figure 30, the sections are cut somewhat obliquely, the two sides of a complete section showing different levels. $\times 25$.

Landacre to the extent that the first rootlet of the portio minor may contain sensory elements from the profundus ganglion. But the first rootlet also contains motor fibers that pass directly into the lateral motor column. The mesencephalic V tract may contain sensory fibers, but sensory fibers entering through the first rootlet of the portio minor may possibly all enter the spinal V tract. Johnston ('05, '09), who argues for the sensory nature of the radix, says that it passes out of the brain by the sensory



root, portio major. This is certainly an error, for both in *Squalus* and *Scyllium* (van Valkenburg) it passes by way of the first rootlet of the portio minor. The portio minor is pre-eminently the motor root of the trigeminus in the selachians. Though the material used by the writers does not warrant the statement that there are no sensory elements in the radix, it does lend support to the view that the radix is an efferent tract, at least in part. As Allen ('19) has recently concluded, the radix mesencephalica trigemini is possibly concerned functionally with the muscle sense.

3. *The ramus ophthalmicus profundus V*

The profundus nerve leaves its ganglion slightly posterior to the distal tip of the latter, on its dorsolateral border (fig. 10). Leaving the ganglion, it swings out dorsolaterally in a gradual curve through the orbit anteriorly, passing ventral to the dorsal rectus muscle (figs. 5 to 7), grazing the mesial wall of the eyeball, again approaching the ramus ophthalmicus superficialis VII so as to pass along the ventral border of the latter close to the skull (fig. 15). Anterior to the emergence of the trochlearis nerve the profundus separates from the facial superficial ophthalmic, passes ventral to the dorsal oblique muscle (fig. 15), then rising dorsally, at the level of the anterior wall of the eyeball passing through a long narrow canal in the cranial wall, it emerges on the dorsal side of the skull, just dorsal to the olfactory bulb (figs. 35 and 51). It divides within the canal into two portions, and on emerging is distributed to the skin of the snout dorsally and laterally. In its course from the ganglion to its final distribution the ophthalmicus profundus gives off three branches, the posterior two of which are the anterior and posterior ciliary nerves (figs. 21, 22, 35, and 51, *cila.*, *cilp.*).

As the ramus ophthalmicus profundus leaves its ganglion there may be recognized at its lateral border a small but distinct bundle of fibers, which may be traced back within the ganglion (figs. 6 to 11, *cilp.*). At no place does this small bundle appear to be closely associated with the other fibers of the nerve. Near the level of the exit of the oculomotor nerve, as the profundus nerve is passing ventral to the dorsal rectus muscle and near the posteromesial border of the eyeball, this small strand of fibers separates spatially from the main nerve (fig. 6, *cilp.*). The fibers of this small strand are all well medullated. At the point of emergence from the profundus, however, non-medullated fibers appear as a distinct tract. In some specimens these fibers can be traced back into the ganglion. On leaving the profundus nerve the non-medullated fibers pass ventrally and posteriorly to join the ciliary plexus (figs. 6 to 8, *cilrl.*). Their subsequent course will be described in the account given later

of the sympathetic system. The medullated fibers are distributed to the wall of the eyeball, passing into the interior by small foramina through the sclerotic cartilage. Although diverse in character and distribution, these fibers given off from the posterior region of the ramus ophthalmicus profundus may be termed the posterior ciliary nerve. The medullated ones seem to represent the ciliares longi in part.

At the level where the trochlear nerve is passing ventrally around the ramus ophthalmicus superficialis VII the ramus ophthalmicus profundus running a little ventral to the other nerves gives off a small branch distributed to the external membranous portion of the sclerotic coat, a small twig passing internally through the cartilaginous portion (figs. 35 and 51, *cila.*). This anterior branch may be termed the anterior ciliary nerve. Between the anterior and posterior ciliary nerves a very minute twig is sent from the r. oph. prof. into the sclerotic coat. The branches of the profundus distributed to the eyeball appear to be the equivalent of the long ciliary nerves of higher forms.

Slightly anterior to the origin of the anterior ciliary nerve another branch leaves the ramus oph. prof. It runs anteriorly across the dorsal oblique muscle on the ventrolateral face of the latter, then at the anterodorsal border of the same muscle turns mesially and dorsally, and at the anteromesial border of the foramen by which the r. oph. spf. VII passes to the dorsal border of the skull, runs by its own small foramen to the dorsal side of the cranium. Passing anteriorly, it is distributed to the skin lateral to the supraorbital canal.

The ramus ophthalmicus profundus in *Mustelus* on entering the orbit gives off a posterior ciliary nerve, also a ciliary branch that unites with the ventral division of the oculomotorius. It then passes through the Y-shaped fork of the rectus internus muscle, meeting the oculomotorius passing in the reverse direction. Anteriorly the profundus passes at the ventral border of the rectus internus, becoming closely applied to the mesial wall of the eyeball. It gives off an anterior ciliary nerve that enters the eyeball on its anterior border. At the level of the origin of the dorsal oblique muscle it passes through the posterior part of

the levator palatoquadrati muscle and enters a foramen in the lateral wall of the cranium, passing into the cranial cavity. At the level of the posterior wall of the nasal capsule it enters a foramen of exit in the dorsolateral wall of the cranium, emerging just ventral to the supraorbital lateral-line canal, lateral to the ramus oph. spf. VII. As it runs anteriorly in this position it divides first into two main branches, then into smaller ones farther on. As described by Allis, most of its terminal branches curve ventrally and then posteriorly around the anterior wall of the nasal capsule, supplying the skin on the ventral side of the snout. The distribution of the profundus in *Mustelus* is thus in sharp contrast to that in *Squalus*, since in the latter the final terminations are dorsal and lateral, reaching anteriorly to the tip of the snout.

4. *The ramus ophthalmicus superficialis V*

Under this designation are included certain small nerves of somatic sensory composition that have a common central origin and a special peripheral distribution, i.e., to the skin dorsal to the orbit. Under this heading we may recognize in *Squalus* three groups of fibers: 1) in a cross-section of the head immediately anterior to the thin anterior part of the profundus ganglion there is seen a small but distinct band of fibers on the ventral border of the ramus ophthalmicus superficialis VII, in size less than one-fifth of the profundus nerve (fig. 10, *os. V 1*); 2) in the same section there may be seen on the mesial dorsal border of the same nerve trunk a smaller band (*os. V 2*); 3) dorsolateral to the r. oph. spf. VII may be seen one or more small nerves (*os. V 3*). Proximally the fibers of 1) may be followed in the angle between the ramus oph. spf. VII (ganglion) and the profundus ganglion (figs. 11, 22, 32, and 35). As the profundus root fibers emerge from their ganglion, the two nerves run side by side, the superficial ophthalmic fibers dorsal in position, but so completely merged are the two nerves that it is difficult in many instances to distinguish between them. But as previously stated, the fibers of the r. oph. spf. V, which at first lie

dorsomesial to the profundus fibers, shift their position so as to run ventral, and enter the dorsomesial part of the gasserian ganglion. Distal to the level of the profundus ganglion this first division of the ramus oph. spf. V. runs at first along the ventral border of the r. oph. spf. VII, then shifts to its lateral border. The final distribution of the terminal branches is around the ventral edge of the supraorbital crest of the skull to the skin dorsal to the eyeball (figs. 5 to 9, 15 to 17). The fibers of 2) may be traced proximally, soon curving ventrally around the mesial border of the r. oph. spf. VII to join the fibers of 1). The two nerves do not always unite, but fibers of 1) may pass into 2) and the reverse. The fibers of 2) end in a mass of large cells on the dorsal border of the gasserian ganglion. Distally 2) passes anteriorly between the r. oph. spf. VII and the lateral cranial wall, and rising to the dorsal border of the nerve trunk divides into a few small branches that pass along with lateral line branches of the r. oph. spf. VII through the supraorbital crest of the cranium to the top of the head, where they are distributed to the skin dorsally, mesial and lateral to the supraorbital canal. If any fibers of this second division of the r. oph. spf. V remain with the main trunk of the r. oph. spf. VII, they become indistinguishably blended with its branches. A number of small nerves, which from their distribution must be regarded as functionally a part of the r. oph. spf. V, pass into the anterior end of the gasserian ganglion in the vicinity of the entrance of nerves 1) and 2). Peripherally, they are distributed to the skin dorsal to the orbit. They pass into special cell masses on the lateral border of the gasserian ganglion (figs. 7 to 11, 22, 31, 32, and 35, *os. V* 3, *gos. V*). The distribution of the ramus ophthalmicus superficialis V in its entirety is on the dorsal side of the head posterior to the area of distribution of the ramus ophthalmicus profundus.

In one specimen a few fibers were found to join intracranially the extreme anterodorsal tip of the gasserian ganglion, just anterior to the root of the portio minor (figs. 21, 22, and 35). Traced peripherally, the fibers are found to pass anteriorly and dorsally intracranially, then through a canal in the anteromesial wall of

V merit especial consideration. The writers reserve this topic for future discussion, believing that only by a comparative study of many elasmobranch forms can any satisfying conclusions be reached in this particular subject.

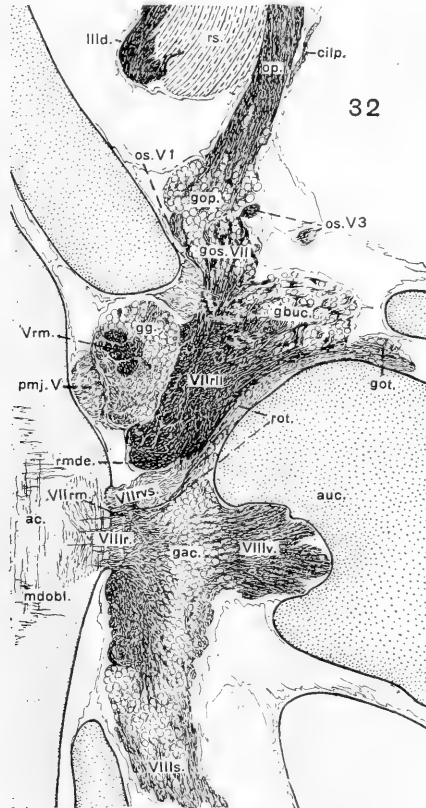


Fig. 32 A horizontal section through the V-VII-VIII ganglia of the right side, showing the distinct root of the ramus oticus VII. $\times 25$.

The ramus ophthalmicus superficialis V of *Mustelus* arises, as previously stated, from a distinct intracranial ganglion. It passes out of the skull as a large compact nerve on the ventral border of the r. oph. spf. VII. It accompanies the latter anteriorly, sending off several small branches that combine with

small branches of the latter. Anteriorly it is distributed to the skin of the dorsal side of the head, dorsal and anterior to the eye (fig. 23).

In those amphibians in which somatic sensory fibers are associated with the i. oph. spf. VII, they occur in a diffuse arrangement, and are given off from the ganglion or near the base of the supraorbital trunk, in a fashion suggestive of the mode of occurrence in *Squalus*.

5. *The ramus maxillaris V*

This nerve arises from the smaller extracranial portion of the gasserian ganglion as a large mass of fibers that joins the ramus buccalis VII to form the infraorbital trunk. At first somewhat triangular in cross-section (figs. 7 to 10 and 20, *mx.*) on the mesial border of the infraorbital trunk, farther anteriorly it becomes a flattened band curved around the ventromesial border (figs. 5, 6, 21, and 22). Still farther anteriorly it covers the infraorbital trunk dorsally, mesially, and ventrally (fig. 24), thence separating into a dorsal, a mesial, and a ventral band. With the giving off of certain small branches to the ventral surface of the head anterior to the mouth, some of which run posteriorly from their origin from the main nerve, the ramus maxillaris together with the ramus buccalis divides into three groups of nerves, the largest mesial one running anteriorly and supplying the ventral surface of the snout mesially and anterior to the level of the eyes. The other two divisions supply the ventrolateral epithelium of the snout. One of the small posteriorly directed branches runs far back at the lateral angle of the mouth, terminating at the lateral border of the oral epithelium between Meckel's cartilage and the palatoquadrate bar (fig. 35, *mxph.*). This small nerve evidently corresponds to Cole's ('96) pharyngeal branch of the ramus maxillaris in *Chimaera*.

The association of the maxillaris with the buccalis is everywhere very intimate, even into the small branches. The large nerves commonly break up into their smaller divisions long before the latter leave the vicinity of the main nerves (figs. 1 to 4 and 34). In consequence their representation in the illustrations is somewhat diagrammatic.

6. *The ramus mandibularis V*

This ramus of somatic sensory and visceral motor fibers derives its sensory elements mostly from the mesial end of the gasserian ganglion. Most of its ganglion cells are intracranial. Its fibers pass directly laterally through and around the posterior border of the maxillary part of the ganglion, and bending posteroventrally around the dorsolateral border of the palatoquadrate bar break up into two divisions, a large dorsal motor branch supplying the adductor mandibulae muscle, and a ventral division that divides into an anterior branch supplying the skin of the ventral side of the lower jaw anteriorly, and a posterior branch that innervates the first ventral constrictor muscle and supplies the skin ventral to this muscle (figs. 15 to 17, 20 to 22, 24, 33, 35, 51 to 53, *md.*). The anterior part of the first ventral constrictor muscle is innervated exclusively by the ramus mandibularis V, but in the posterior part where its fibers mingle indistinguishably with those of the second constrictor muscle, there is evidently innervation also from the truncus hyomandibularis VII. This ventral motor branch of the mandibularis also innervates a part of the adductor mandibulae.

From the dorsal border of the main trunk of the ramus mandibularis shortly after leaving the ganglion there are given off a few (three or four) small branches, which break up into numerous small twigs, motor elements supplying the levator palatoquadrate and spiracular muscles and sensory fibers supplying the skin dorsolaterally in the region of the spiracle (fig. 35, *lp-q.*). From the main mandibular trunk, as it is passing around the lateral border of the palatoquadrate bar, there is given off from its ventral side a small nerve. This follows the main nerve around to the ventral border of the bar of cartilage, but as the mandibular trunk turns posteriorly this small nerve bends anteroventrally along the lateral border of the palatoquadrate bar, entering the lateral border of the posterior part of the pre-orbital muscle, and furnishing the innervation of the latter (figs. 35 and 51, *pro.*). The writers do not find in any of the many series of sections examined any anastomosis between the ramus

mandibularis and the ramus maxillaris such as Landaere describes. But during the progress of this research attention was called to a laboratory dissection of an adult *Squalus* in which there is a large anastomosis between the ramus mandibularis and the infraorbital trunk. It arises from the ramus mandibularis, as the latter is passing around the palatoquadrate bar, and runs anterolaterally, then anteromesially, over the preorbital muscle across the floor of the orbit to join the infraorbital trunk

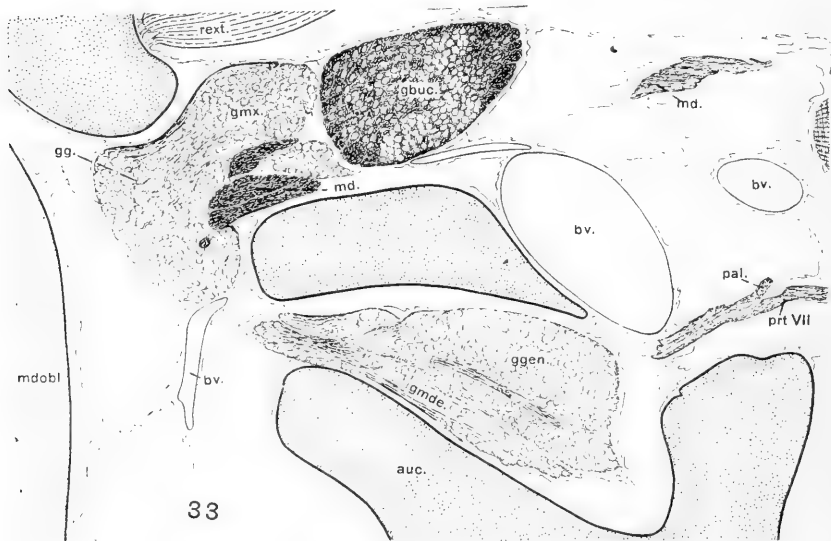


Fig. 33 A horizontal section through the trigemino-facial foramen, showing chiefly the gasserian and geniculate ganglia. $\times 30$.

at the region where the latter breaks up into its chief large branches destined to the skin and lateral-line organs. It seemingly joins a maxillary branch, and probably consists of somatic sensory fibers that belong primarily to the ramus maxillaris. As the ramus maxillaris is leaving its ganglion it gives off a minute twig which passes ventrolaterally around the ventral border of the ramus buccalis VII out to the ramus mandibularis V as the latter is passing around the lateral border of the palatoquadrate bar. The small nerve in question does not unite with

the ramus mandibularis, however, but passes along the anterior border of the latter, thence ventrally, laterally, and anteriorly, to the base of the eyelid, then turning posteriorly it passes along the internal border of the jugular group of ampullae of Lorenzini, becoming smaller and smaller as it is distributed to the cutaneous tissue (fig. 35, *mxpc.*).

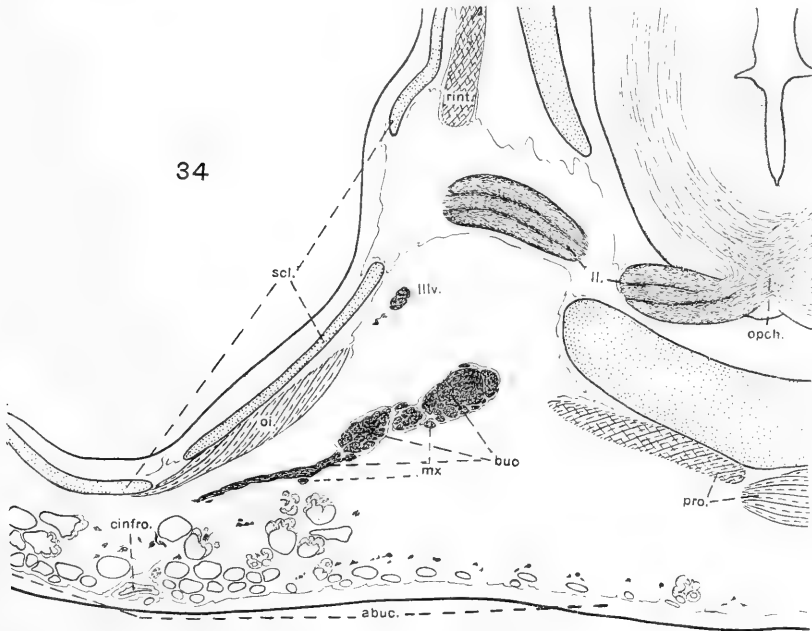


Fig. 34 A cross-section through the optic chiasma, showing structures in the angle between the ventrolateral brain wall and the ventromesial wall of the eyeball. Section 692. $\times 15$.

THE FACIAL NERVE

1. The lateral-line roots and ganglia of the facial nerve

The roots of the lateral-line component of the facialis enter the medulla in two divisions: a dorsal root passing into the lateral-line lobe and a ventral root entering the acusticum (figs. 36, 12 to 14, 21, 22, 35, and 51, *VIIrlld.*, *VIIrllv.*). A horizontal

section through the dorsal root at its point of entrance into the brain shows that its fibers pass into the brain substance on each side of a vertical internal furrow or sulcus in the lateral-line lobe (fig. 39), in this way spreading fan-shape in the brain wall. In a similar fashion the ventral root fibers spread anteriorly and posteriorly in the acusticum (figs. 25 to 30). According to Strong ('03), the dorsal root is formed by the union of fibers from the truncus hyomandibularis and ramus buccalis, but the ventral root fibers are derived from the ramus ophthalmicus superficialis VII and pass through the dorsal root in order to reach the acusticum. Cole ('96) and Cole and Dakin ('06) state that in *Chimaera* the rami ophthalmicus superficialis, buccalis and hyomandibularis each connects with the brain by a dorsal [lateral-line lobe] and a lateral [acusticum] root. Allis ('01) states that in *Mustelus* the dorsal (superficial ophthalmic) and ventral (buccal-hyomandibular) roots of the 'trigeminus II' [lateral-line component of the facialis] each connects with the 'lobus trigemini' [lateral-line lobe] and with the tuberculum acusticum. This account of Allis the writers corroborate, but would add that in *Mustelus* both the hyomandibularis and the buccalis send fibers into the lateral-line lobe and also into the acusticum. In brief, the relations in *Mustelus* are exactly like those in *Chimaera*. It seems highly improbable that the conditions in other elasmobranchs are different from those in *Mustelus* and *Chimaera*. In *Squalus* the interweaving of the root fibers makes it well nigh impossible to determine with accuracy their peripheral distribution, although from a study of *Squalus* material alone the writers would agree with Strong. As Cole and Dakin state for *Chimaera*, so for *Mustelus* it may be said that the truncus hyomandibularis sends but few fibers into the acusticum.

Hawkes ('06) states that in *Chlamydoselachus* the hyomandibular trunk arises from the brain at the same level as the root of the trigeminus and facialis proper, and that the superficial ophthalmic VII and buccal arise by two roots situated more dorsally. It seems to the writers more probable that the so-called hyomandibular roots in *Chlamydoselachus* are the visceral sensory and motor, and that the 'rami communicantes'



Fig. 35 A projection upon the sagittal plane of the chief rami of the trigeminal and facial nerves. To show the distribution of the somatic sensory elements of the trigeminal nerve through the rami ophthalmicus profundus, ophthalmicus superficialis and maxillaris. Sensory elements in the mandibular branches innervating the levator palato-quadrati and spiracularis muscles are also shown (*lvp-q.*). Somatic sensory elements are shown in black. $\times 8$.



Fig. 36 A cross section through the roots of the facial and auditory nerves of the right side. Section 998. $\times 25$.

origin of the rectus lateralis (externus) muscle, is crescentric, dumb-bell shaped, an anterodorsal superficial ophthalmic portion and a posteroventral buccal portion, the two parts being

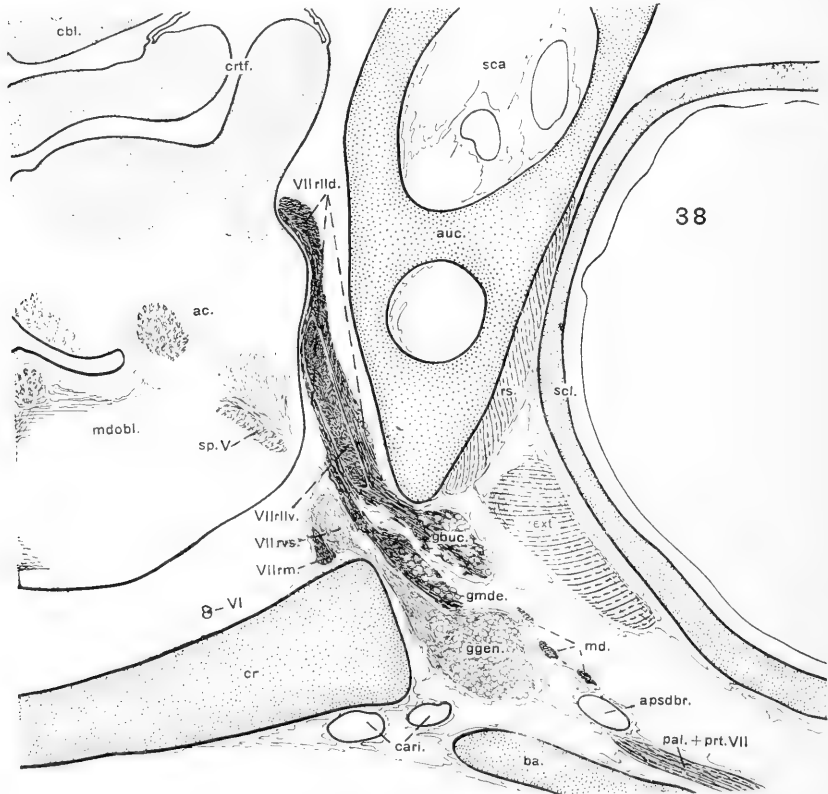


Fig. 38 A cross-section through the roots of the left facial nerve of *Mustelus californicus*, slightly anterior to that shown in the preceding figure. The dorsal lateral-line root in its passage ventrally is seen 'straddling' (to use the expression of Allis) the ventral lateral-line root. The section cuts the posterior end of the buccal ganglion (*gbuc.*) and the anterior end of the ganglion of the ramus mandibularis externus VII (*gmde.*). $\times 25$.

continuous through a narrow ganglionic neck (figs. 15 to 17, 21, and 24, *gos. VII*, *gbuc.*). The anterior portion, from which arises the ramus ophthalmicus superficialis VII, extends forward some distance as a central core in the superficial ophthalmic

trunk, being covered externally by nerve fibers (figs. 5 to 11). Where the two parts of the ganglion are confluent, fibers predominate in the ophthalmic portion. The ventral (posterior) or external mandibular ganglion is situated out on the truncus hyomandibularis (figs. 20, 24, 33, and 51, *gmde.*). It is a round column of cells that does not appreciably modify the form of the nerve trunk. Its root fibers run in toward the brain dorsal to the root of the geniculate ganglion and ventral to the vestibular division of the auditory nerve (figs. 17, 31, 32, and 36, *rmde.*), and turning dorsally around the anterior border of the vestibular part of the auditory ganglion (figs. 12, 21, 22, and 24), join the main lateral-line roots.

The superficial ophthalmic and buccal portions of the dorsal ganglion in *Mustelus* are not confluent, as in *Squalus*, but widely separated. Both are extracranial (fig. 23).

The shape of the anterior ganglion (oph. spf. and buc.) in *Squalus* is modified by certain groups of cells that almost merit the designation as distinct ganglia. At the dorsolateral border of the buccalis portion of the anterior lateral-line ganglion, just ventral to the ampulla of the anterior semicircular canal, is a small conical collection of cells protruding from the general mass (fig. 35, *gbuc. a.*). From this there pass out three small nerves (only two shown in fig. 35) that supply fourteen ramuli to the neuromasts of the postorbital section of the infra-orbital canal. A little farther posteriorly (figs. 32 and 35), on the extreme posterior border of the lateral-line ganglion, a more conspicuous mass of cells (*got.*) extends in a fashion similar to the preceding. From it there pass out through a canal in the lateral portion of the ear capsule to the top of the skull fibers that supply the first five or six ramuli to the neuromasts of the main lateral-line canal of the trunk. From its origin and distribution this latter nerve must be regarded as the equivalent of the ramus oticus of various authors. Besides innervating the lateral-line canal, it supplies a peculiar tubular organ on the anterior median border of the spiracle (fig. 20, *ospr.*). Its root fibers can be traced into the acusticum by a small bundle that enters the brain immediately posterior to the visceral sensory root of the facialis (fig. 32, *rot.*).

In *Mustelus* there is a large posterodorsal extension of the buccal ganglion within the orbit, from which a number of small nerves emerge, one of which, from its distribution to the posterior horizontal portion of the infraorbital canal, Allis correctly considers as the equivalent of the ramus oticus. The rest of the small nerves from the otic portion of the ganglion supply the postorbital section of the infraorbital canal and the spiracular organ which in *Squalus* is innervated by the ramus oticus proper. In *Mustelus* the hyomandibular trunk does not lie in a canal in the ventral wall of the ear capsule, but in the ventral portion of the orbit. The ganglion of the ramus mandibularis externus VII is not such a long slender column of cells as in *Squalus*, confined to the narrow hyomandibular canal, but lying free in the ventral part of the orbit assumes a more globular form (figs. 23 and 37, *gmde*).

2. *The roots and ganglia of the facialis proper*

The geniculate ganglion is situated upon the hyomandibular trunk in the larger proximal part of the hyomandibular canal, in the cranial wall ventral to the vestibular portion of the ear (figs. 17, 24, and 33, *ggen.*). The geniculate ganglion is everywhere distinct from other ganglia, even where in contact with them. From the ganglion proximally the visceral sensory root fibers, accompanied by groups of motor fibers, pass into the cranial cavity, and curving around the anterior ventral border of the auditory ganglion, between its cells and the root of the ventral lateral-line ganglion, enter the medulla and pass directly to the visceral sensory column in the latter (figs. 12 to 14, 26 to 29, 31, 32, and 36, *VII rvs.*). The slightly medullated character of its fibers renders them especially easy to distinguish from other fiber tracts. The motor root passes through the brain substance immediately ventral to the sensory fibers, from the periphery to the lateral motor column. Some of the motor root fibers have the appearance of arising in the ventral motor column. Within the brain the sensory root divides, one part passing directly into the visceral lobe, there to curve posteriorly along

the latter; the other part passes posteriorly some distance before entering the visceral lobe (figs. 26 and 27). Within the brain these two roots, sensory and motor, lie ventral to the ventral-line root and dorsal to the anterior border of the great auditory root (figs. 22 and 36). Outside the brain, accompanying and dorsal to the motor and visceral sensory roots of the facialis, is the posterior (ventral) lateral-line root, whose ganglion is situated on the hyomandibular trunk mostly distal to the geniculate ganglion (fig. 24).

3. *The ramus ophthalmicus superficialis VII*

This large nerve is related to the anterior part of the dorsal (anterior) lateral-line ganglion. As already stated, the ganglion is so overgrown with fibers that few of the cells appear on the exterior, but for the most part form a core that extends far out in the nerve (figs. 5 to 11, 15 to 17, *gos. VII*). The nerve passes anteriorly through the dorsal portion of the orbit, giving off small nerves that pass dorsally through small foramina in the supra-orbital crest of the cranium to supply supra-orbital canal organs (fig. 50, *os. VII*). Rising higher in its anterior course the main nerve at the anterior part of the orbit passes by its own large foramen through the crest of the cranium to the top of the head, where it courses anteriorly supplying supra-orbital canal organs and the supra-orbital group of ampullae of Lorenzini. The superficial ophthalmic has few large branches. The largest one of these, given off shortly after the main nerve emerges from the cranium, runs anteroventrally to supply the canal organs situated in that part of the supra-orbital canal that runs from the tip of the snout posteroventrally around to the ventral side of the head to join the infra-orbital canal (figs. 35, 50 to 52, *eth.*). Anteriorly the main nerve breaks up into numerous small branches that supply canal organs and ampullae of Lorenzini (figs. 1 to 4, *os. VII*). Accompanying the superficial ophthalmic VII are two of the divisions (*os. V 1* and *os. V 2*) of the superficial ophthalmic V. These are so closely applied to the facialis ramus as to appear like branches of the latter when given off.

Their destination has been described. Arising from the ganglion near the base of the r. oph. spf. VII are a few small much elongate nerves that innervate the posterior part of the supraorbital canal. Branches of the r. oph. spf. V (*os. V 3*) are closely associated with some of them.

4. *The ramus buccalis VII*

The intimate association of this ramus with the ramus maxillaris V to form the truncus infra-orbitalis has been described. Its distribution is to the sense-organs of the infra-orbital canal and the associated ampullae of Lorenzini. From the ganglion near the base of the ramus buccalis are given off a few small nerves (fig. 50). One of these supplies the anterior portion of the main lateral-line canal. Its distribution and relations will be discussed under the head of the ramus oticus VII. The other small nerves just mentioned collectively innervate the canal organs of the postorbital section of the infra-orbital canal. The ramus buccalis proper gives off few branches until it breaks up, with the ramus maxillaris, into three main branches or rather, three loose collections of small branches. Some of these supply ampullae of Lorenzini only, but the most of them are distributed to canal organs and ampullae indifferently.

In *Mustelus*, as Allis has shown, the maxillary and mandibular rami run for some distance with the ramus buccalis in a common infra-orbital trunk. In this trunk the maxillary and mandibular sensory elements are completely merged, but always distinct from the buccalis.

5. *The ramus oticus VII*

The ramus oticus, as mentioned in a preceding section, enters a distinct ganglionic mass on the posterior distal part of the buccal ganglion. The root fibers, however, unlike those of the buccalis, seem to connect with the acusticum only, and not with the lateral-line lobe. The canal organs which it supplies lie in that part of the canal system which is the immediate posterior continuation of the supra-orbital canal, between the latter

and the section of the canal innervated by the glossopharyngeus. About five ramuli are contributed to canal organs (fig. 50, *ot. VII*)

Hawkes finds a ramus oticus in *Chlamydoselachus* that supplies the postorbital section of the infra-orbital canal, and the horizontal section of the infra-orbital canal (anterior end of the main lateral-line canal), and also sends two small branches to the skin. These latter she interprets as general cutaneous. But comparison with *Squalus* would suggest that in *Chlamydoselachus* these cutaneous branches may possibly innervate spiracular organs as in *Squalus*, or isolated neuromasts, pit-organs.

Besides innervating canal organs in *Squalus*, the ramus oticus supplies a peculiar tubular organ on the anterior mesial wall of the spiracle (fig. 20, *ospr.*). This is apparently a modified ampulla of Lorenzini.

Landacre describes in the 22-mm. embryo a lateral-line primordium which he regards as corresponding to the most posterior sense-organs of the infra-orbital canal. This receives two small branches from the buccalis ganglion, arising in the angle between the buccalis and the r. oph. spf. VII. He describes a second small twig arising more posteriorly, which innervates a lateral-line primordium that he believes gives rise to lateral-line organs near the junction of the supra-orbital and infra-orbital canals. He considers this the ramus oticus auctorum. He notes that this ramus after supplying twigs to the lateral-line primordium sends a branch more posteriorly, ending in the anterior wall of the spiracle.

As stated in a preceding section, the ramus oticus VII in *Mustelus* arises from a ganglionic mass in common with the small nerves that innervate the postorbital section of the infra-orbital canal. An account of the occurrence, innervation, and homologies of the spiracular organ of elasmobranchs, ganoids, and dipnoans has been given elsewhere by the writers ('20).

Herrick ('99) considers the ramus oticus of *Menidia* as primitively a general cutaneous dorsal ramus of the facialis to which

lateralis elements have been added secondarily. In *Squalus*, *Mustelus*, and *Raja*, and presumably in all elasmobranchs, it is wholly lateralis.

6. *The truncus hyomandibularis VII*

There are three chief constituents of the hyomandibular trunk: *a*) lateral line, *ramus mandibularis externus*, whose root, as already described, enters the lateral-line lobe and the acusticum together with the buccalis root, and whose ganglion occurs mostly distal to the geniculate ganglion as an elongated column which scarcely affects the size or shape of the hyomandibular trunk (figs. 17, 20, 24, 48, 51 to 53, *mde.*, *gmde.*); *b*) visceral sensory, *ramus mandibularis internus*, whose fibers arise in the geniculate ganglion (figs. 48, 51 to 53), *mdi.*); *c*) visceral motor, *ramus hyoideus*, the motor constituent of the facial nerve (figs. 48, 51 to 53, *hy.*).

Emerging from the hyomandibular canal, the hyomandibular trunk passes posterodorsally without branching, at the lateral border of the ear capsule, around the mesial dorsal border of the spiracle to the lateral border of the anterior part of the second dorsal constrictor muscle and at the lateral border of the hyomandibular cartilage. Thence it passes posteroventrally along the anterior lateral border of the same muscle and posterior border of the spiracle (figs. 48, 51 to 53, *tr. hmd.*).

In *Mustelus* the lateralis component of the hyomandibularis is relatively smaller than in *Squalus*, due probably to the lack of the hyoidean group of ampullae of Lorenzini in the former.

The ramus mandibularis externus VII. The first branch of considerable size given off from the hyomandibular trunk is the anterior division of the *ramus mandibularis externus*. It leaves the main trunk at the horizontal level of the ventral end of the hyomandibular cartilage and runs anteroventrally, dividing into a dorsal branch that supplies the jugular or hyomandibular canal and a ventral branch that innervates the mandibular canal (fig. 50). After giving off the last twig to the mandibular canal organs, this ventral branch sends a twig into a tubular structure

that contains a well-developed sense-organ in its posterior end. The tubular organ in question lies anteroventrally to the mandibular canal, opening to the exterior at its own anteroventral end. It is about 0.7 mm. in length. It is apparently a modified ampulla of Lorenzini. From this tubular organ the anterior branch of the ramus mandibularis externus continues in an anteroventral direction, in close proximity to the anterior sensory division of the ramus mandibularis V, and finally comes to a series of tubular organs ventral to the lateral border of Meckel's cartilage, between the latter and the skin. These tubular organs all open upon the mucous membrane, or near the line of junction of the mucous membrane with the epidermis at the lateral angle of the mouth. In the single specimen in which they were carefully examined they are eleven in number, and are evidently the rudiments of a group of ampullae of Lorenzini. All these tubular organs are innervated by the nerve branch mentioned above.

The main part of the posterior division of the ramus mandibularis externus supplies the hyoidean group of ampullae. It passes posteroventrally from the point of division as a stout nerve that suddenly breaks up into its terminal twigs for the rosette of hyoidean ampullae. From the base of the posterior division of the ramus mandibularis externus there runs anteriorly along the lateroventral border of the adductor mandibulae muscle a small nerve whose destination is a series of pit-organs in the jugular region, lying mostly ventral to the hyomandibular canal. There are about twenty-five of these pit-organs. Farther dorsally is another series of pit-organs innervated by small twigs from the main hyomandibular trunk before the branching of the latter. There are about eight pit-organs in this second group. The two groups constitute a single series almost in the shape of a semicircle, extending from the spiracle nearly to the midventral line.

Hawkes finds in *Chlamydoselachus* three branches of the ramus mandibularis externus which seem to correspond in a general way to the dorsal and ventral branches of the anterior division and the entire posterior division in *Squalus*. She notices several branches

which are interpreted as somatic sensory. It may be suggested that microscopical examination will show that these supposed general cutaneous nerves end in pit-organs or similar structures, as in *Squalus*.

Cole ('96) describes in *Chimaera* "two groups of ampullae, situated behind the lower jaw," and innervated by the *ramus mandibularis externus*. These ampullae are small and simple in structure, and probably correspond to the small rudimentary ampullae and the near-by larger tubular organ in *Squalus*, innervated by branches of the *ramus mandibularis externus VII*.

The ramus mandibularis internus VII. Slightly posterior and dorsal to the origin of the anterior division of the *ramus mandibularis externus* from the hyomandibular trunk the *ramus mandibularis internus* is given off (figs. 48 and 51), and as the visceral sensory terminal branch of the hyomandibular trunk passes transversely around the lateral edge of the hyoid cartilage, into the interval between the latter and Meckel's cartilage, and turning anteriorly runs along the dorsomesial border of the latter between it and the hyoid cartilage, as far as the anterior end of the latter. Thence it continues anteriorly between the ventrolateral edge of the basihyal and Meckel's cartilage, now dividing into a number of small branches. Its area of distribution is the lower jaw and the ventrolateral floor of the mouth mesial and anterior to the chorda tympani.

The ramus hyoideus VII. As the hyomandibular trunk passes along the lateral border of the second dorsal constrictor muscle, it gives off numerous small twigs to the muscle (fig. 49). Farther ventrally the remaining motor fibers separate from the lateral-line elements, which form the posterior branch of the *ramus mandibularis externus*, at the lateral border of the hyoid cartilage, as described above. Thence as a compact nerve they pass ventrally around the hyoid cartilage to the ventral side of the lower jaw, and turning anteriorly between the first and second ventral constrictor muscles are distributed to the second ventral constrictor and the posterior part of the first constrictor.

7. The ramus palatinus VII

From the enlarged proximal portion of the geniculate ganglion there passes out through the wall of the hyomandibular canal ventrolaterally a nerve of visceral sensory composition, that on emerging turns anteroventrally soon dividing into an anterior and a posterior branch (figs. 21, 22, 48, and 51, *prt. VII, pal.*). The anterior division passes anteriorly, situated between the base of the cranium and the palatoquadrate bar, dorsal to the roof of the mouth. It continues anteriorly in this position, giving off branches to the dorsal oral epithelium: ramus palatinus VII. The posterior branch passes posteriorly at the mesial ventral border of the palatoquadrate bar, giving off branches to the dorsolateral oral membrane, until the level of the anterior wall of the spiracle is reached. Here it turns ventrally and anteriorly and runs along the dorsolateral surface of Meckel's cartilage, farther anteriorly breaking up into small branches supplying the ventrolateral oral surfaces—the so-called chorda tympani. From the geniculate ganglion slightly posterior to the point of origin of the ramus palatinus, there are given off two or three small nerves that supply the pseudobranch and the anterior wall of the spiracle (figs. 21, 22, 48 and 51). These have been termed ramus pretrematicus by various authors.

8. General reflections upon the facial nerve

Excluding the lateral-line elements, the facial nerve exhibits the three characteristics of a branchial nerve: ramus pharyngeus (palatinus), ramus pretrematicus (so-called chorda tympani) and ramus posttrematicus (hyomandibularis). The hitherto so-called rami pretrematici are to be regarded as twigs that belong to the palatinus and pretrematicus proper, but are given off separately. The anastomoses that occur between these so-called pretrematic rami and the ramus pretrematicus proper support the view of their common origin. The rami hyoideus and mandibularis internus may be regarded as the visceral motor and visceral sensory constituents of a ramus posttrematicus. Divergent opinions have been expressed as to the homol-

ogies of these rami of the facialis. Regarding the ramus palatinus in a narrow sense, there can be little question. The so-called chorda tympani is plainly prespiracular or pretrematic and does not correspond to the chorda tympani of higher forms.

The ramus alveolaris or mandibularis internus of amphibians is variously interpreted as pretrematic or posttrematic. Drüner ('01, '04), who has exhaustively studied this subject, regards the ramus alveolaris of the urodele amphibians as pretrematic. In that case the posttrematic VII in amphibians lacks a visceral sensory element. In Siren (Wilder, '91; Norris, '13) the palatine and alveolar rami arise from the ganglion by a common trunk, as do the palatine and pretrematic rami in *Squalus*. In most amphibians the ramus alveolaris leaves the geniculate ganglion in the hyomandibular trunk, in some separately as an independent nerve. In its distribution the ramus mandibularis internus of *Squalus* resembles more closely the ramus alveolaris of amphibians, i.e., it runs along the mesial border of the lower jaw (or even within the dentary bone in amphibians), while the ramus pretrematicus of sharks runs along the lateral border. In considering the homology of any nerve its peripheral distribution as well as its origin should be taken into consideration, as Strong ('90, '92) has so clearly pointed out. May it not be that the ramus alveolaris of amphibians is a combined nerve, i.e., a fusion of pretrematic and posttrematic visceral sensory elements? Herrick ('99), indeed, suggests "that the amphibian ramus mandibularis internus VII may represent both pre- and postspiracular communis elements."

The facial nerve proper in *Squalus*, and presumably in all sharks, is completely free from anastomoses with other nerves. Jacobson's commissure, ramus communicans X ad VII, anastomoses with the ramus ophthalmicus profundus V or other parts of the trigeminal nerve, are wholly absent. General cutaneous elements are absent both in root and branches. It is a typical facial nerve, containing only visceral sensory and visceral motor components.

The double condition of the ramus mandibularis externus VII is possibly to be regarded as foreshadowing the condition in the

amphibians, where it divides into rami mentalis externus and internus. Or if the condition in *Chlamydoselachus*, as described by Hawkes, is primitive, the amphibian condition may have been derived from that.

Four varieties of lateral-line sense-organs occur in *Squalus*: canal organs (neuromasts in canals), pit-organs (naked neuromasts in the skin), ampullae of Lorenzini, and certain peculiar tubular organs (on anterior wall of spiracle, on lower jaw near mandibular canal), probably modified ampullae.

THE AUDITORY NERVE

The root fibers of the auditory nerve pass from the ganglion into the acusticum as a single large, broad root, slightly posterior to the facialis roots (figs. 12 to 14, 31, 32, and 36, *VIIIr.*). It is well-nigh impossible from a study of cross-sections alone to draw a sharp line of distinction between the auditory fibers and the ventral lateral-line root. But in sagittal and horizontal sections they are seen to be distinct (figs. 27 to 30, *VIIIr.*, *rliv.*). On entering the acusticum the auditory root is seen to divide into anteriorly and posteriorly directed tracts, the posterior tract being the larger (fig. 28, *VIIIr.*).

The auditory ganglion at its anterior vestibular end is large and globular, but posterior to the root fibers the ganglion cell mass assumes the form of a vertical plate, though farther posteriorly it becomes a rounded column, the saccular ganglion (figs. 12, 31, and 32). The anterior end of the auditory ganglion is wholly intracranial; but gradually passing out laterally and posteriorly through the cranial wall, its posterior portion becomes situated in a recess in the ventral part of the ear capsule. Near the anterior end of the ganglion a large vestibular nerve passes into the ear capsule, supplying the utriculus and the ampullae of the anterior and horizontal semicircular canals. Succeeding this large vestibular branch there follow numerous branches dorsally and ventrally supplying the sacculus and finally the ampulla of the posterior canal (figs. 12, 21, 31, and 32).

THE GLOSSOPHARYNGEAL NERVE

1. The roots and ganglia in the glossopharyngeal nerve

The ganglion of the ninth nerve is farther removed from the brain wall than that of any other cranial nerve. It is situated in the ventrolateral part of the ear capsule, near the posterior end of the latter, in a large chamber hollowed out of the cartilage. Its root, composed of lateral-line, visceral sensory, and visceral motor fibers, passes anteriorly and mesially from the ganglion to its entrance into the medulla at a point about opposite the middle portion of the spiracle. At the anteromesial end of the visceral sensory ganglion and extending mesially upon the root, is a small mass of cells, the ganglion of the lateral-line component (figs. 24 and 48, *gspt. IX*). The visceral sensory root enters the medulla and passes anteromesially into the visceral lobe. The lateral-line component enters the brain immediately dorsal to the anterior part of the visceral root, ventral to the extreme anterior end of the lateral-line root of the vagus nerve (fig. 24). Within the brain the lateral-line root of the ninth nerve runs anteriorly ventral to the mesial border of, and nearly parallel with, the root fibers of the lateralis X, but always distinct from them. Anteriorly, the lateral-line element of the ninth approaches that of the tenth, and finally takes a position at the mesial border of the other lateral-line tracts, just lateral to the visceral lobe. The motor elements in the glossopharyngeal root occur in scattered bundles. In similar fashion they connect with the brain by a number of minute rootlets, in position anterior to the other roots.

2. The lateral-line elements in the glossopharyngeal nerve

Various writers have recognized lateral-line elements in the ninth nerve of fishes, although their presence there has been generally overlooked or ignored. Allis ('89, '97) describes and figures a lateral-line component of the glossopharyngeus of *Amia*, innervating one canal organ and a line of pit-organs. Ewart ('92) and Ewart and Cole ('95) found a lateral-line component in the

ninth nerve of *Laemargus*, which innervates "three sense organs of the lateral line lying immediately posterior to the commissural canal," besides sending branches to the skin. It may be suggested that these latter branches possibly supply pit-organs, as in *Amia*. In *Chimaera* Cole ('96) finds that from the ninth "ganglion a very fine dorsal branch is sent up to the skin, but this does not innervate any sense organs of the lateral line." Hawkes ('06) describes a small ganglion upon the root of the glossopharyngeal nerve in *Chlamydoselachus* from which a small dorsal branch proceeds, innervating two neuromasts of the lateral line, besides sending twigs to the skin. Allis ('01) is not certain of the presence of this element in the ninth nerve of *Mustelus*. Johnson ('17) figures various stages in the development of *Squalus acanthias*, showing an unnamed dorsal branch of the ninth nerve. In the 22-mm. stage of *Squalus Landacre* ('16) finds in the glossopharyngeal nerve a lateral-line ganglion related to two primordia and a branch of uncertain distribution. Stannius ('49, p. 79) finds in *Spinax acanthias* and *Carcharias glaucus* a dorsal branch whose origin, course, and distribution answer to the dorsal lateral-line ramus of the ninth in *Squalus*. He fails to find such a ramus in the rays, although a fine nerve in *Raja batis*, a branch of the glossopharyngeus, was traced to the region of the external acoustic pore. The writers confirm in part the statement of Stannius that no dorsal lateral-line branch of the ninth nerve occurs in the rays. In *Raja radiata* there is no lateral-line element in the ninth nerve, nor any dorsal branch of the latter. Van Wijhe ('82) states that in stage K of the embryo of *Scyllium* the ninth nerve differentiates into a dorsal and a ventral ramus, of which the former unites with an epidermal thickening which is the anlage of that portion of the canal-organ system innervated by the glossopharyngeus. In stage L the glossopharyngeal ganglion begins to divide into a ganglion at the base of the dorsal ramus and another on the ventral ramus. Klinkhardt ('05) finds in embryos of *Spinax niger* a dorsal cutaneous connection on the glossopharyngeus. Wright ('85) mentions a dorsal lateral-line branch of the ninth nerve in *Mustelus*. Pansch ('10) describes a lateral-line component in the

ninth nerve of Lota. Herriek ('01) describes a lateral-line component in the glossopharyngeus of *Ameiurus melas*, derived, however, from the vagus, which supplies one canal organ and two pit-organs. The writers find in *Mustelus californicus* a small lateral-line ganglion upon the ninth nerve in essentially the same position as the one in *Squalus*, from which a nerve proceeds dorsally, contributing two ramuli to the lateral-line canal. In one specimen examined a single ramulus occurs.

Cole ('98) says that the glossopharyngeus takes "no part in the innervation of the system [lateralis] either in *Gadus* or morphologically in any other form." Kingsbury ('97) finds a lateralis constituent in the ninth of *Amia*, but derived from the main lateral-line nerve. Allis ('97) says that the lateral-line element in the glossopharyngeal nerve of *Amia* is derived from the root of the lateral-line nerve of the vagus, but in the larva bears a distinct ganglion.

3. *The ramus supratemporalis IX*

From the small lateral-line ganglion on the root of the glossopharyngeus all the fibers pass dorsoposteriorly as a single minute nerve, through a short canal in the lateral wall of the ear capsule, to the dorsal side of the head. Thence running anteriorly, they are distributed through three terminal ramuli to that part of the main lateral-line canal between the distribution areas of the ramus oticus VII and the ramus supratemporalis X. No pit-organs are connected with the ninth nerve, nor are somatic sensory elements found in it. Houser ('01) mentions and figures general cutaneous fibers in the ninth nerve, and also states their occurrence in the tenth nerve, of *Mustelus canis*.

4. *The first branchial nerve*

The glossopharyngeal nerve in *Squalus* may be taken as an example of a typical branchial nerve. It consists of four rami: lateralis, pharyngeal, pretrematic, and posttrematic. The posttrematic, however, is itself distinctly double. All but the lateralis contain visceral sensory elements, the posttrematic dif-

fering from the pharyngeal and pretrematic in possessing visceral motor fibers in its main portion.

Within its canal in the ear capsule the ninth nerve emerges from the posterior end of the visceral ganglion in two portions, a smaller lateral visceral sensory part, and a large mesial portion of sensory and motor composition (figs. 24, 48, 51, and 52). On emerging from the canal in the ear capsule, the smaller portion divides into two chief branches at the ventromesial border of the thymus gland. The mesial one of these divisions turns anteriorly along the roof of the pharynx and mouth dorsally: ramus pharyngeus IX (figs. 48, 51 and 52, *ph. IX*). The lateral branch of the lateral sensory division of the glossopharyngeus turns antero-ventrally from its point of separation from the mesial branch and runs along the anterior wall of the first gill cleft—ramus pretrematicus IX (figs. 48 and 51, *prt. IX*).

After giving off the rr. pharyngeus and pretrematicus, the remaining fibers of the ninth nerve constitute a large nerve lying dorsal to the mesial end of the first epibranchial cartilage. Along the anterolateral face of this cartilage it runs ventrally, curving at first posteriorly and then ventrally as it shifts over to the first ceratobranchial cartilage. It runs along the dorso-lateral border, later along the ventrolateral border of this cartilage. Shifting to the mesial border, it runs to the extreme anterior tip of the cartilage and farther anteriorly at the dorso-lateral border of the coracobranchialis muscle, beyond the anterior end of which it runs at the dorsomesial border of the posterior horn of the basihyal. Farther anteriorly it passes along the dorsal border of the lateral portion of the main part of the basihyal cartilage, supplying the mucous membrane of the floor of the pharynx and mouth. Besides the chief portion of the ramus posttrematicus there are some secondary parts given off as small visceral sensory branches (fig. 48, *pst. IXa*) that run more directly anteroventrally across the anterolateral face of the epibranchial cartilage, thence along the ceratobranchial bar a little dorsal to the main ramus. The general course of the ramus posttrematicus IX is thus seen to be along the mucous membrane of the posterior wall of the first gill-cleft.

THE VAGUS NERVE

1. The lateral-line roots and ganglia of the vagus nerve

The roots of the vagus nerve in their entirety when seen in a lateral view (figs. 24, 48, and 51) form a wedge-like sheet of fibers on the side of the medulla. Converging at the postero-ventral angle of the wedge, the combined fibers form a compact column that produces a hollowing out of the mesial wall of the ear capsule and forms a recess, the beginning of a canal or chamber in the extreme posterior portion of the ventromesial part of the ear capsule. This chamber in which the combined lateral-line and visceral sensory ganglia for the greater part lie, opens posteriorly at the border of the occipital condyle.

As indicated in a previous section, the lateral-line element in the ninth nerve enters the brain immediately anterior and ventral to the anterior lateral-line roots of the vagus (figs. 24 and 51). Within the brain the vagus lateral-line fibers may be traced far anteriorly as a wedge-shaped (in cross-section) tract reaching from the periphery of the brain inward as far as the lateral border of the visceral lobe. On entering the brain the lateral-line fibers of the vagus all turn anteriorly (fig. 40). Farther anteriorly the tract appears to be made up of scattered bundles and finally becomes indistinguishable from the other medullated tracts of the acusticum. Peripherally, the root fibers form a broad ribbon-like band lateral to the brain, covering the anterior roots of the vagus proper lying mesially. Farther posteriorly, the lateral-line root drops ventrally so that the more posterior roots of the vagus are exposed. Still farther posteriorly and ending in the ganglion, the root becomes more compact covering merely the ventral border of the vagus roots (figs. 24, 48, and 51).

The lateral-line ganglion of the vagus shows evidence of a segmentation into three parts. The first is the more anterior part of the vagal ganglionic complex, a slender column of cells that gives rise to the supratemporal ramus. Just posterior to this first cell mass and for some distance in contact with it lies the major part of the ganglion, two scarcely separable masses of cells, from the anterior of which arise the fibers of the ramus

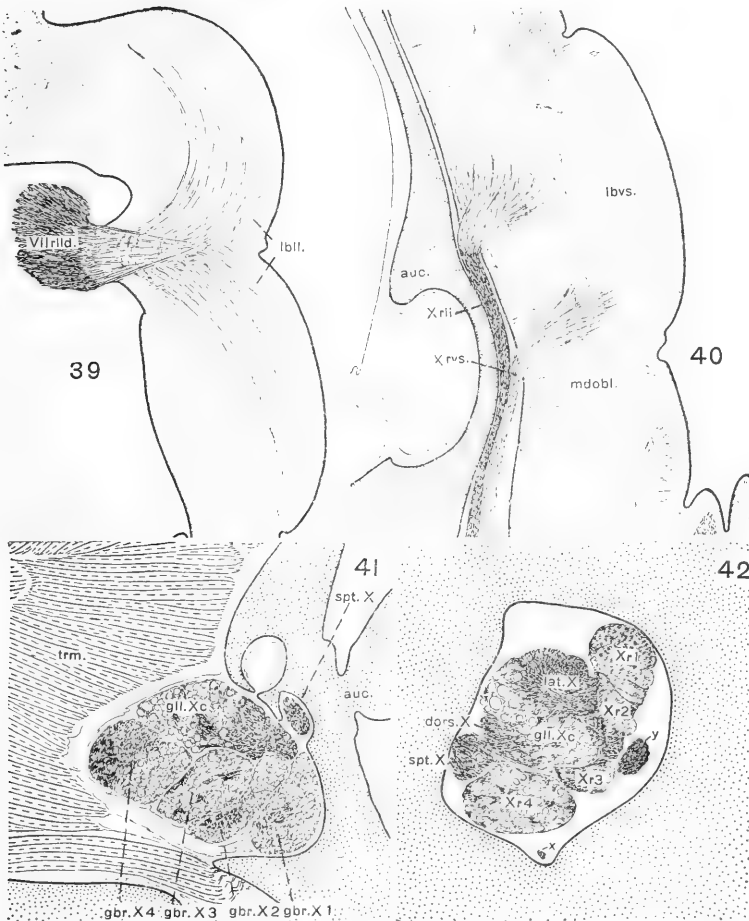


Fig. 39 A horizontal section through the dorsal lateral-line root of the facial nerve. $\times 30$.

Fig. 40 A horizontal section through the lateral-line root and a visceral sensory root of the vagus nerve, showing that the lateral-line root fibers all pass anteriorly on entering the brain. A composite of three sections. $\times 25$.

Fig. 41 A parasagittal section through the right vagal ganglionic complex, showing the segmental arrangement of the branchial ganglia. $\times 31$.

Fig. 42 A cross-section through the roots of the right vagus nerve, showing the distinctness of the individual roots and ganglia. Section 1279. $\times 31$.

dorsalis. Fibers from the third ganglion together with the remaining fibers from the second form the ramus lateralis of the trunk. In a horizontal section through the ganglionic complex (fig. 45) there are seen external swellings indicating these three ganglia. In a sagittal section the ganglion of the supratemporal ramus is seen to be anatomically distinct from the others.

In the 22-mm. embryo Landacre finds three lateral-line ganglia upon the vagus. From the first the supratemporal ramus emerges connecting with three lateral-line primordia; from the second ganglion a ramus, evidently ramus dorsalis, connects with a large lateral-line primordium, and from the third ganglion proceeds the ramus lateralis.

In *Mustelus* the ganglion of the supratemporal ramus is anatomically distinct from the other vagal ganglia, not even in contact with them. A similar condition occurs in *Raja radiata*. In *Mustelus* the ganglion is sometimes very much elongate, partly intracranial and partly extracranial.

2. The roots and ganglia of the vagus nerve proper

The visceral sensory roots of the vagus extend for a long distance posterodorsally from the origin of the lateral-line roots (figs. 24 and 51). There are thirty or more of the visceral roots of the vagus. The majority of them are mixed, each comprising a wide sensory band and a small motor element. The posterior six or more are exclusively motor in composition. The extreme posterior three or four rootlets arise from the dorsal side of the medulla. Figure 43 is of a parasagittal section through the lateral wall of the medulla cutting most of the vagus rootlets. It will be seen that these enter the brain in an almost straight line, rising gradually from anterior to posterior. As the rootlets enter the brain, the motor element in each of the mixed rootlets is usually ventral in position. As they pass inward the sensory elements ascend into the visceral lobe, and the motor fibers turn ventrally into the lateral motor column. At the brain wall there is little evidence of a segmental arrangement

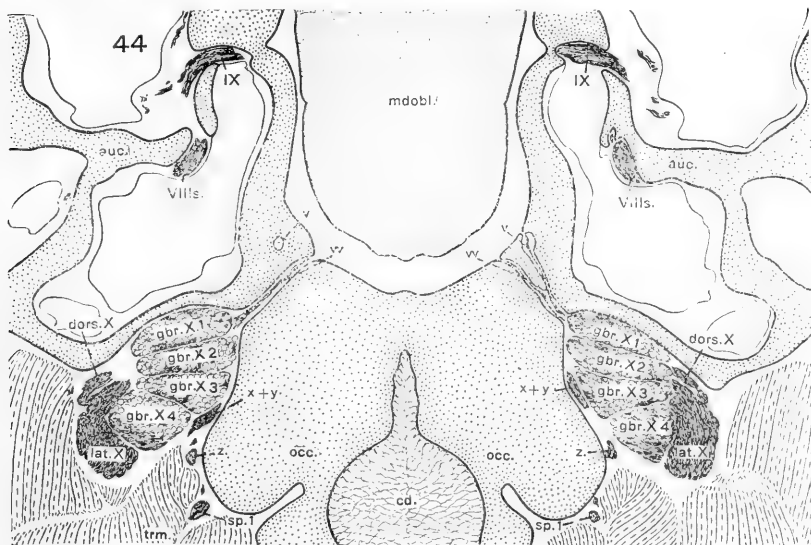
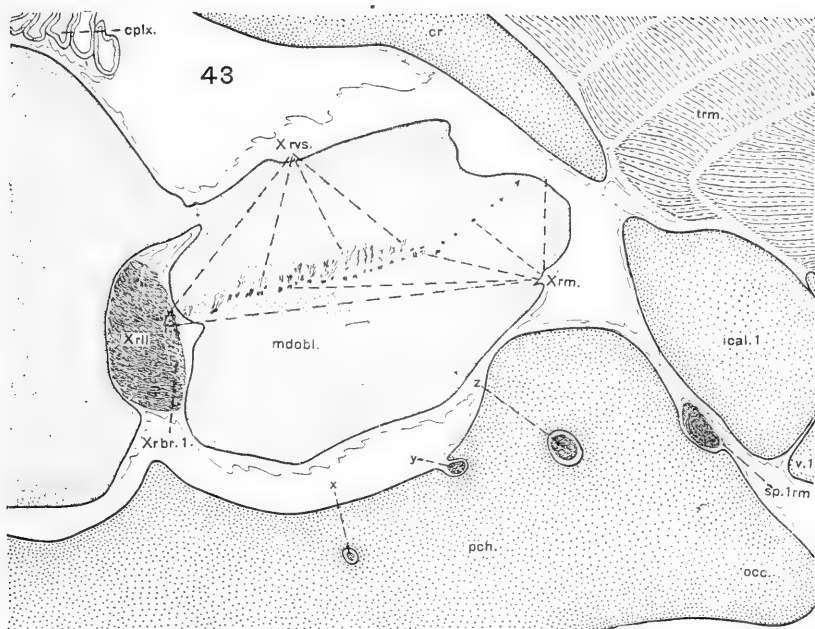
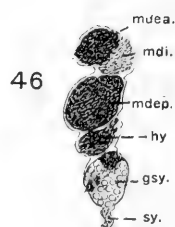
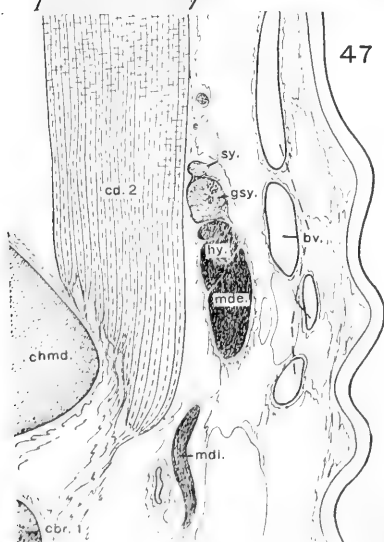
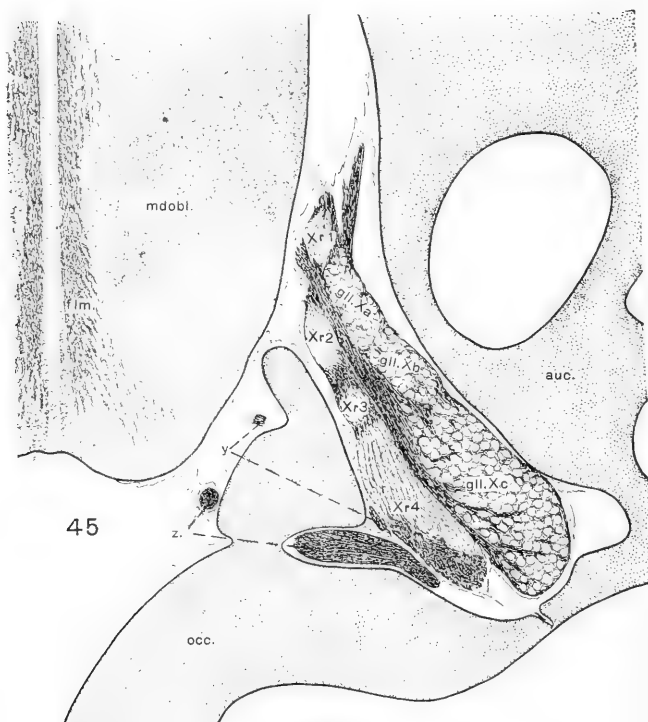


Fig. 43 A parasagittal section through the left lateral border of the medulla oblongata, cutting the rootlets of the vagus nerve. Four groups of rootlets are shown somewhat indistinctly, corresponding to the roots of the four branchial nerves. $\times 25$.

Fig. 44 A horizontal section through the ear capsules and parachordal region of the cranium, showing five occipital nerves and the segmental arrangement of the four vagal branchial ganglia. A composite of three sections. $\times 15$.



of the roots. Figure 43 shows that the arrangement of the rootlets is in an almost uninterrupted series from anterior to posterior. But in the sheet of fibers at the lateral border of the medulla, formed by these rootlets, the individual branchial nerves soon begin to differentiate, and in cross-section the segmental character of this sheet-like root of the vagus becomes very evident (fig. 42). The four branchial and the single intestinal rami become differentiated successively anteriorly posteriorly, the last branchial and the intestinal, in the roots as well as peripheral to the ganglion, being closely related. Without doubt there is individual variation in the arrangement of the rootlets. In a study of cross-sections it is often difficult to distinguish between the individual rootlets, but comparisons between cross-sections and parasagittal sections shows that the variations in the roots of the vagus are very few indeed.

The visceral vagal ganglionic mass shows a segmentation into four branchial and one intestinal divisions, all more or less in contact, and the last two closely fused. Horizontal and parasagittal sections show very clearly this segmental arrangement (figs. 41 and 44). The common ganglion of the fifth branchial and the intestinal nerve is larger than the others and plainly double. Proximal to this last ganglion and related to it there are two distinct roots. One the branchial root, connects with the ganglion more anteriorly and ventrally than does the intestinal root. Distally there emerge from the ganglion two nerves, a ventral branchial and farther posteriorly a dorsal intestinal. The motor fibers that pass through this fourth ganglion mass belong to the intestinal division and are of two kinds: coarse ones to the trapezius muscle, and finer, less medullated ones to the wall of the digestive tract.

Fig. 45 A horizontal section through the roots of the right vagus nerve, showing the four vagal branchial roots and the segmented condition of the lateral-line ganglion. $\times 30$.

Fig. 46 A section of the left truncus hyomandibularis VII, cut horizontally through the head, but obliquely to the nerve, showing the components of the truncus and a sympathetic ganglion on the posterior border of the nerve. $\times 30$.

Fig. 47 A section of the left truncus hyomandibularis VII, cut transversely through the head, but obliquely to the nerve, showing the components of the nerve and a sympathetic ganglion on the posterior border of the nerve. Corresponds approximately to section 1188 in figure 48. $\times 30$.

3. *The ramus supratemporalis X*

This nerve is exclusively lateralis in composition. It leaves the ventrolateral border of the anterior division of the lateral-line ganglion, and after running posteriorly for some distance in the vagal canal passes out dorsolaterally through a small canal in the posterior wall of the ear capsule (figs. 41 and 42, *spt. X*). It divides within the canal, an anterior branch passing dorsally and anteriorly along the dorsolateral border of the ear capsule and finally to the periphery to supply the sense-organs of the commissural canal and the two pit-organs situated just anterior to the external opening of the endolymphatic duct (fig. 50, *spt. X*). A posterior branch passing dorsally and posteriorly supplies ramuli to the neuromasts of the main lateral-line canal immediately following those supplied by the ramus supratemporalis IX. In the specimen used in the plotting of figure 51 there are six of these ramuli supplied to the main canal; in a second specimen only four.

4. *The ramus dorsalis X*

The term ramus dorsalis is here used as a non-committal designation of a ramus exclusively lateralis in composition, that arises from the second of the three divisions of the lateral-line ganglionic mass of the vagus, and is distributed in part to neuromasts in the main lateral-line canal, but mostly to pit-organs in the occipital and dorsal anterior trunk regions. It evidently comprises a number of nerves, which, however, have a common origin from the ganglion and a similar distribution. There emerge from the ventrolateral border of the ganglion three bands of fibers. The two anterior may unite loosely into a common nerve, the third runs almost directly posteriorly and soon separates from the other two, rejoining them only through anastomoses. A large tract of one of the anterior bands may rejoin the main lateral-line nerve. There is a tendency in the origin of all of them for small tracts of fibers from the ganglion to join the larger ones some distance from the origins of the latter, forming in this manner a kind of plexus. A single nerve finally

results from the plexus, extending posteriorly nearly to the first dorsal fin.

Fibers of the ramus dorsalis are distributed through a variable number of nerve ramuli to the section of the main sensory canal immediately posterior to that innervated by the ramus supratemporalis X. In one specimen there are eight of these ramuli; in a second specimen eleven. It should be noted, however, that in the first one the ramus supratemporalis X has six ramuli, and in the second four. That is, there seems to be a sort of reciprocal arrangement of ramuli between the rami supratemporalis and dorsalis. Besides innervating the sensory canal, the ramus dorsalis supplies a dorsal series of pit organs extending from immediately posterior to the commissural canal nearly to the first dorsal fin. In the specimen from which figure 50 was made there are twenty-five of these pit-organs on the left side. It is a debatable question whether the ramus dorsalis as here defined is a single nerve, but the presence of anastomoses between its divisions precludes any exact separation between them. In *Mustelus* the ramus dorsalis is formed in a similar fashion from two or three small nerves.

5. General cutaneous elements in the vagus nerve of elasmobranchs

It has been quite generally assumed that the vagus nerve of selachians contains somatic sensory elements. The rami supratemporalis and dorsalis have been commonly designated as dorsal cutaneous branches of the vagus. The writers confess to being seriously misled by this general assumption in the early part of this research.

Stannius finds a single dorsal branch of the vagus in selachians, which he regards as lateral-line exclusively. Ewart and Cole believe that the supratemporal branches of both the glossopharyngeus and the vagus of *Laemargus* contain cutaneous as well as lateralis elements. Cole finds in *Chimaera* a dorsal branch of the ninth nerve supplying the skin, but not special sense-organs. A dorsal branch of the vagus, on the other hand, innervates canal organs. Kappers ('14) finds cutaneous con-

stituents in the seventh, ninth, and tenth nerves of *Hexanchus* and *Heptanchus*, but not in other selachians. Houser describes and figures general cutaneous fibers in the vagus nerve of *Mustelus canis*. Landacre describes in the embryo of *Squalus acanthias* a ganglion, which he regards as general cutaneous, on the vagus nerve together with a nerve which he believes to be the equivalent of a *ramus auricularis*. In a figure prepared for the first edition of Herrick and Crosby's *Laboratory Outlines in Neurology* ('18), the writers represented the *ramus dorsalis* of *Squalus* as containing somatic sensory fibers. The true relations are shown in the second edition ('20, fig. 5). Besides fibers terminating in canal organs, there were other constituents of the *ramus dorsalis* that ended in the skin, losing for the most part their myelin near their termination. More careful examination has shown that in every instance such fibers of the *ramus dorsalis* terminate in pit-organs. The writers are forced to the conclusion that in *Squalus acanthias* there is no general cutaneous component in the vagus nerve of the adult and late embryonic condition. Careful search has revealed no connections between the *tractus spinalis trigemini* and the vagus nerve. Nor can there be found any ganglia in the vagus nerve of *Squalus* except the three *lateralis* ganglia and the four (five) ganglia on the branchial and intestinal nerves.

The occurrence of well-defined somatic sensory components in dorsal branches of the vagus nerve in ganoids and teleosts (Herrick, '99, '00, '01; Allis, '97; Panschin, '10) leads to the expectation of finding them in the selachians. It may be that the condition in *Squalus* is not typical. The finding of a somatic sensory ganglion by Landacre in the embryo suggests that its occurrence may be general.

Hawkes notes that two kinds of fibers are found in the dorsal branches of the ninth and tenth nerves of *Chlamydoselachus*: coarse fibers, interpreted as lateral-line, and "smaller medullated fibers probably general cutaneous." The writers have observed that the pit-organs in *Squalus* are innervated by fibers of noticeably smaller caliber and less dense medullation than those supplying the canal organs.

General cutaneous elements in the vagus of amphibians, with the exception of the caecilians (Norris and Hughes, '18), have long been recognized.

6. *The ramus lateralis X*

This large nerve runs out of the posterior ventral border of the lateral-line ganglion. Its fibers are derived from the second and third divisions of the latter. Its fibers are distributed to the canal organs of the main lateral line and to the series of dorso-lateral pit-organs situated dorsolaterally above the lateral-line canal of the trunk, termed by Johnson ('17) the accessory pit-organs (fig. 50).

7. *The second to the fifth branchial nerves*

The branchial nerves derived from the vagus, in their relations to the branchial clefts and arches are essentially similar to the glossopharyngeal nerve in its relations to the structures of the first branchial cleft and arch. Each nerve may be traced to a distinct root far anterior to its own ganglion, suggesting the elongate root of the ninth. Peripherally to the ganglion each nerve divides into three rami: pharyngeal, pretrematic and posttrematic, with distributionssimilar to the corresponding rami of the glossopharyngeus. The posttrematic, consisting of a main and a secondary portion, as in the ninth, with similar courses, innervates the corresponding interarcual, adductor arcus branchialis, interbranchial, and ventral constrictor muscles. The innervation of the adductor arcus branchialis is in each instance by small nerves that pass through foramina in the epibranchial cartilage.

The fifth branchial nerve contains only visceral sensory fibers (sympathetic elements being ignored). It leaves the ganglion common to it and the ramus intestinalis farther anteriorly than the latter. After the separation of the other branchial nerves, the fifth continues its position at the ventral mesial border of the ramus intestinalis until it reaches the transverse level of the extreme posterior border of the fourth adductor muscle, where it gives off a ramus posttrematicus. The latter runs posteriorly

across the dorsal border of the fourth interarcual muscle, then farther ventrally along and across the elongate fifth epibranchial bar, near the ventral end of the latter turning anteriorly to run at the lateral border of the muscular wall of the digestive tract, where it enters a ganglion (fig. 48). From the ganglion several nerves pass anteriorly, apparently distributed to the wall of the pericardial chamber. This anterior end of the ramus posttrematicus forms a so-called ramus cardiacus.

As the fifth branchial nerve is passing across the interarcual muscle a ramus pretrematicus is given off running posteriorly, ventrally, and finally anteriorly along the anterior wall of the fifth branchial cleft. Near the posterior border of the interarcual muscle a ramus pharyngeus passes through the muscle to the roof of the pharynx and has the usual distribution.

Where the main lateral-line ganglion is in contact with the visceral ganglion of the fourth branchial nerve (*X3*), a tract of fibers passes from the former into the latter, thence out of the ganglion along with the motor constituent and through the main fourth branchial nerve into its posttrematic ramus. It continues in the latter around to the ventral part of the fourth branchial arch. On the posterior border of the fourth ceratobranchial cartilage it leaves the posttrematic ramus through a small sympathetic ganglion and passes anteriorly, ventrally, and mesially in the fleshy connections of the fourth branchial arch with the lateral trunk wall. It then turns posteriorly at the lateral border of the coracobranchialis group of muscles, then farther posteriorly runs along the lateral border of the coracoarcualis muscle, and continues in this position, descending ventrally in its course until the posterior lateral border of the pectoral girdle is reached. Here it turns mesially around the pectoral girdle and runs posteriorly in the ventral body wall, breaking up into small twigs distributed to a small group of pit-organs situated posterolaterally to the base of the yolkstalk of the embryo (figs. 48 and 50, *vpo.*).

Of the occurrence in *Squalus* of 'rami pretrematici interni,' such as are stated by Sewertzoff ('11) to be found in *Scyllium*, *Acanthias*, *Raja*, and *Trigon*, the writers find little evidence. Sew-



Fig. 48 A projection upon the sagittal plane of the five branchial nerves and the ramus hyomandibularis VII, to show the situation of the branchial sympathetic ganglia. The small nerve twigs given off from the posttrematic rami are motor branches, most of which contain visceral sensory fibers. $\times 10$.

ertzoff gives no figures or exact descriptions of these nerves in *Acanthias*, and his citation of Bender ('06) as an authority for their occurrence in the latter form is of little weight, since Bender gives neither figures nor description of them in *Acanthias*. After an examination of the figures of Sewertzoff and Bender there can be little doubt of the presence of these so-called rami pretrematici interni in *Heptanchus*, *Scyllium*, *Raja*, and *Centrophorus*, but in *Squalus acanthias* the ramus pharyngeus of the branchial nerves does not divide into a dorsal pharyngeus and a latero-ventral ramus pretrematicus internus. The entire ramus pharyngeus is distributed dorsally. The pharyngeal rami of the third and fourth branchial nerves of the vagus do divide immediately on emerging from the main nerve, but into anterior and posterior divisions, both distributed dorsally and not laterally. Pharyngeal, pretrematic and posttrematic rami occur in all the branchial nerves including the facialis proper. Bender regards the ramus palatinus of selachians as a combination of a ramus pharyngeus with a ramus pretrematicus. In quite similar fashion the ramus pharyngeus of the glossopharyngeal nerve unites for some distance with the ramus pretrematicus. In both the facialis and the glossopharyngeus of *Squalus* the ramus pretrematicus near its dorsal end gives off small twigs, in one case to the pseudo-branch, in the other to the wall of the first gill cleft. In the vagal branchial nerves the pharyngeal and pretrematic rami arise from the main nerve sharply distinct from each other. The accessory posttrematic rami occur in a double condition in the glossopharyngeus and fourth vagal branchial (fig. 48).

8. *The ramus intestino-accessorius X*

As this nerve trunk leaves the ganglion posteriorly, its coarse motor constituents form a narrow band along its dorsal border. These soon separate as a distinct nerve that passes posteriorly along the dorsal border of the visceral sensory elements of the nerve trunk, finally rising in a few divisions into the trapezius muscle which it innervates (figs. 48 and 51, *trap.*). The fine motor and the sensory elements of the ramus continue straight

posteriorly nearly to the posterior border of the fifth branchial arch. Then it divides into three main branches whose subdivisions are distributed chiefly to the wall of the digestive tract.

THE OCCIPITAL NERVES

The occipital nerves in *Squalus acanthias* are variable in number. Two seems to be the more constant number, but in some instances two well-developed and one or more rudimentary ones may be found. In the same head there may occur a difference in number between the two sides. Fürbringer ('97) reports two or three occipital nerves in *Acanthias vulgaris*. Following the method of Fürbringer, the writers designate the occipital nerves from posterior to anterior by the letters z, y, x, etc.

The anterior rootlets of the occipital nerve y may be discerned somewhat anterior to the transverse level of the posterior motor roots of the vagus (fig. 51, y). They arise, in marked contrast to the visceral motor roots of the vagus, from the ventral motor column. About six rootlets contribute to the formation of the nerve. After its formation the nerve passes at once into the vagal canal, sometimes by a distinct foramen. Within the canal (fig. 42, y) it passes back with the vagal complex and divides into a larger ventral and a smaller dorsal portion. The smaller division, in passing out dorsally through a small canal in the roof of the ear capsule (figs. 49 and 51), is accompanied by a corresponding branch of the occipital nerve z, the two being distributed to dorsal trunk muscles. The main portion of the nerve y continues through the vagal canal accompanied by the main part of nerve z. As the two nerves pass into the first interbasal (subspinalis) muscle, or more correctly stated, between its dorsal and ventral portions, they are joined by a ventral branch of the first spinal nerve. This latter nerve contains somatic sensory as well as somatic motor fibers. The three nerves pass back through the muscle and at its lateral border merge into a single trunk, in which, however, the individuality of the contributing nerves is not lost.

Occipital nerve z originates by four or five rootlets from the region where the spinal cord merges into the medulla. It passes at once through a small foramen into the vagal canal, almost immediately on entering giving off the small dorsal branch that passes out dorsally with a corresponding branch of the nerve y. As stated above, it accompanies the preceding occipital nerve posteriorly and finally unites with it and a branch of the first spinal nerve. It gives off small branches to the first interbasal muscle.

The occipital nerve x, when present, is very small. In the single instance in which it was traced from origin to innervation, it was found to leave the brain wall some distance anterior to the exit of y. Passing back ventral to the vagus roots it enters a minute canal in the base of the cranium, merging into the vagal canal, where it runs posteriorly at the ventral border of the branchial ganglia (fig. 42). On leaving the vagal canal, it takes a position at the lateral border of the basal plate, soon joining the nerve y, with which it passes back some distance, finally turning mesially into the mesial portion of the first interbasal muscle, which it innervates. Figure 44 is a horizontal section through the cranial basis of a specimen with five occipital nerves. The destinations of nerves v and w were not traced.

According to Fürbringer, there are usually three nerve branches supplying the interbasal muscles in selachians. One or two of these, derived from y or y and z, innervate the first interbasal (subspinalis). One or two branches derived from y and z supply interbasals 2-3. The writers find in *Squalus acanthias* five nerve branches supplying the first interbasal muscle: one derived from nerve x, two from y, one from z, and one from the first spinal nerve. That the other interbasal muscles are innervated as Fürbringer describes is probable, but the writers are unable to confirm his statements.

The small foramen of exit of the nerve z into the vagal canal is designated by Wells ('17) as a secondary foramen of the vagus nerve.

THE FIRST THREE SPINAL NERVES

The more anterior motor rootlets of the first spinal nerve appear a short distance posterior to the last rootlets of the occipital nerve z, at a level slightly posterior to that of the posterior border of the roof of the cranium. The motor root thus formed passes out at once through a foramen between the first vertebra and the occipital condyle (fig. 43, *sp.1rm.*). On emerging, it divides into a ventral and a dorsal ramus, the latter passing posteriorly and then anteriorly around the antero-lateral border of the first spinal ganglion, apparently without receiving any sensory elements from it, and gives off branches to the dorsal trunk muscles. The ventral motor ramus, after sending off one or more branches that run posteriorly in the ventral portion of the dorsal trunk muscles, is joined by a sensory ramus from the first spinal ganglion, the two forming a nerve which running posteriorly at the lateral border of the occipital condyle joins the two occipital nerves as previously described (fig. 49).

The sensory root of the first spinal nerve enters the dorsal portion of the spinal cord somewhat posterior to the posterior rootlets of the motor root. Its course from the spinal cord to its foramen of exit through the first intercalary cartilage is posteriorly directed, then anteriorly into the ganglion situated immediately outside the foramen. From the extreme posterior end of the ganglion a branch runs ventrolaterally to join the ventral motor ramus as described above. One or more (usually two) sensory nerves connect with the anterior end of the ganglion (fig. 49).

The anterior rootlets of the second spinal nerve begin to emerge as far anteriorly as the level of the sensory root of the first spinal nerve. Numerous rootlets converge to form a root that passes out at the level of the posterior rootlets through a foramen in the first vertebra, dividing immediately on exit into a dorsal and a ventral branch. In the number and arrangement of its parts the second spinal nerve is almost a duplication of the first spinal. Its ventral ramus of motor and sensory composition runs posteriorly at the lateral border of the first and second ver-

tebrae, and just posterior to the origin of the third spinal nerve fuses with the trunk formed by the union of the occipital and first spinal nerves, forming what is known as the cervical plexus (figs. 49 and 51). It will be seen that this account of the main branches of the anterior spinal nerves in *Squalus acanthias* is not wholly in agreement with the description given by Allen ('17) of the corresponding parts of a posterior abdominal spinal nerve in the same species.

The form and branches of the third spinal nerve repeat with little variation those of the two preceding nerves. The ventral ramus passes out through a foramen in the second vertebra, and after running posteriorly some distance comes into intimate relations with the combined mass of the ventral rami of the occipital and first and second spinal nerves. Only with extreme care can it be followed in sections through the cervical plexus. It is seen to receive a motor branch from the second spinal nerve, but all its own motor fibers pass into the brachial plexus. It sends anteroventrally one or more large sensory branches closely paralleling the great hypobranchial nerve formed from the cervical plexus (figs. 49, 51, and 53). This account is in close agreement with that of Fürbringer for *Acanthias*, but he states that in some instances the third spinal nerve contributes to the cervical plexus a sensory branch.

Müller ('11) states that in *Acanthias vulgaris* there are three occipital nerves, later in development two. In seventeen examples he finds that the third spinal nerve forms the first brachial nerve; in six instances it is the second spinal, and in four instances, the fourth spinal.

THE HYPOBRANCHIAL NERVE

The great nerve trunk formed by the union of the ventral rami of the occipital and first two spinal nerves is composed of somatic sensory and somatic motor fibers. Visceral sensory and motor fibers, if present, are not in evidence. Careful examination of cross-sections of the nerve shows that it is possible to distinguish with considerable accuracy the parts contributed by the various

nerves. As the nerve trunk approaches the place of its separation into the chief branches of distribution, these elements become more distinct. The derivative of the third spinal nerve retains its individuality throughout, and never actually unites with the others. The sensory elements of the spinal-nerve constituents are coalesced and become intimately associated with the occipital-nerve derivatives. The motor elements of the first and second spinal nerves are distinct.

In this condition the hypobranchial nerve forms a flat horizontal band at the ventral border of the dorsal trunk muscles, mesial to the ramus intestinalis X. Gradually it shifts laterally dorsal to the ramus intestinalis, and finally, passing ventrally around the lateral border of it, breaks up into its chief branches. There are three of these branches, two directed anteriorly and one posteriorly. In the readjustment of the constituent elements to form the peripheral divisions, the sensory elements from the first and second spinal nerves combine with the motor constituents of the occipital and second spinal nerves to form a trunk that sends a small motor branch posteriorly into the third spinal nerve and thence into the brachial plexus, and a large branch anteriorly along the mesial dorsal border of the limb-girdle (fig. 49). When the posterior end of the fifth epibranchial cartilage is reached, the nerve runs along its mesial border, then shifting to the ventral border of the fifth ceratobranchial bar and dividing into two or three divisions that later reunite, it finally unites with the second anterior division of the hypobranchial nerve, just outside the ventrolateral wall of the pericardium, on the dorsal border of the coraco-arcualis communis muscle, which it innervates. In its course along the border of the coraco-arcualis muscle the motor hypobranchial trunk divides into a dorsal and a ventral branch. The latter, the larger, turns sharply through the muscle ventrally, sends branches into the coraco-mandibularis and enters and passes anteriorly within the coraco-hyoideus muscle. The dorsal branch runs along the dorsomesial border of the coraco-arcualis communis, and after passing through this muscle, between the first and second divisions of the coracobranchialis muscle, is distributed to the latter.

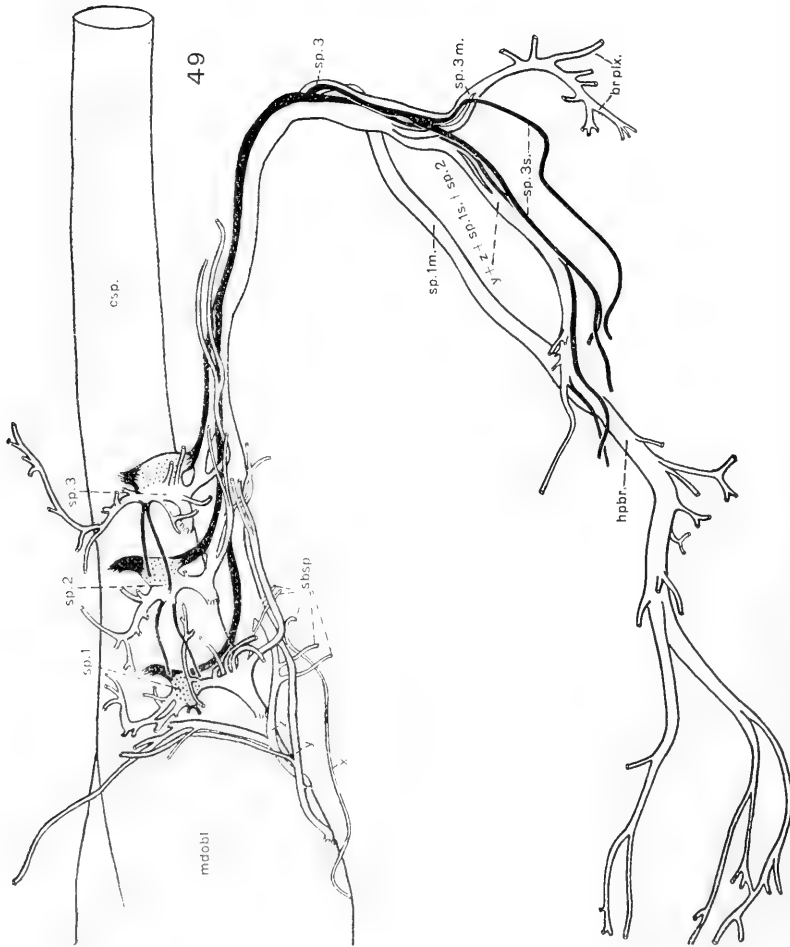


Fig. 49 A projection upon the sagittal plane of the hypobranchial nerve, its origin, composition, and distribution. The sensory elements are shown in black. $\times 10$.

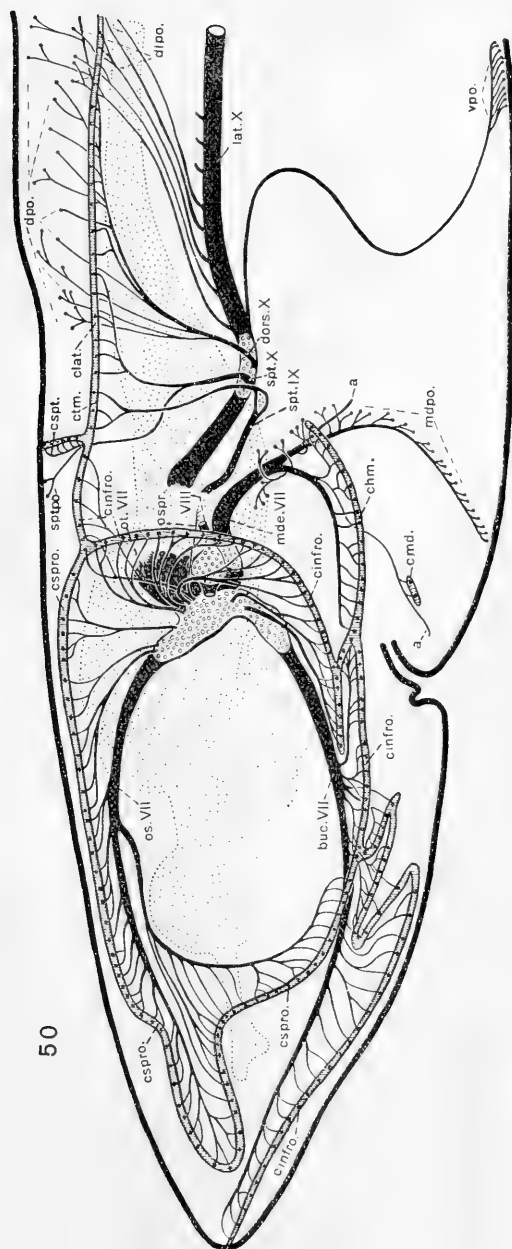


Fig. 50 A somewhat schematic projection upon the sagittal plane of the lateral-line canals, the pit-organs, and their innervation. The infraorbital canal on the ventral side of the head is represented as swung ventrally out of its natural horizontal position. The number of nerve ramuli entering the canals, the number and position of the pit-organs, and the number and arrangement of the nerve twigs ending in the pit-organs are represented approximately correctly. For the most part the ampullae of Lorenzini are ignored.

The second division of the hypobranchial nerve, derived from the motor constituent of the first spinal nerve, runs anteriorly somewhat dorsal to the first division. At first it passes along the lateral border of the esophagus, just outside its muscular wall, farther anteriorly ventral to the lateral part of the pharynx, then at the mesial dorsal border of the fifth ceratobranchial cartilage, and still farther anteriorly ventral to it. It then unites with the first branch, as already described. At the union of the two nerves apparently all of the sensory elements of the first division have been given off in minute nerves to the ventral and ventrolateral skin in the branchial and postbranchial region, and from this point onward the hypobranchial nerve is exclusively motor. Anterior to the shoulder girdle, the motor hypobranchialis innervates the coraco-arcualis communis, coracomandibularis, coracohyoideus, and coracobranchiales muscles.

Neal ('97) derives the hypoglossal musculature of *Squalus* from the fourth to the eighth postotic somites. The second and third postotic somites are present; the second is rudimentary, and the third is without a ventrally growing bud, but with a nerve. From the fourth somite comes the coracomandibularis muscle, apparently. The fifth to the eighth somites produce muscles between the hyoid and procoracoid cartilages. The seventh and eighth somites also contribute to the pectoral plate. If one takes into account the occurrence of as many as five pairs of occipital nerves in the pup stage, it is seen that there is slight discrepancy between the account given by the writers and that of Neal relating to an earlier stage.

According to van Wijhe, the hypoglossus nerve is derived from three (apparently four) ventral roots of the seventh, eighth, and ninth somites, on the last of which is a sympathetic ganglion. In *Squalus* the writers find a small sympathetic ganglion on the ventral root of the third spinal nerve.

Individual variation in the derivatives of the somites and their corresponding nerves probably explains the minor discrepancies between the accounts of various writers.

Comparing the hypobranchial nerve of selachians with the hypoglossal nerve of amphibians, we see some suggestive re-

semblances. The hypoglossal nerve of the urodele amphibians is formed from the ventral branches of one or more spinal nerves. In some forms, as *Siren*, sensory elements are contributed at the origin of the nerve, to be given off before the true hypoglossal nerve is reached. In the caecilians sensory elements are likewise found at the origin of the nerve, and in some species of caecilians an occipital nerve forms part of the hypoglossal. The ventral musculature supplied by the hypobranchial nerve in selachians undoubtedly corresponds in a general way to the ventral musculature anterior to the shoulder-girdle in amphibians. In *Siren*, for example, where the hypoglossus is derived from two or three spinal nerves, it innervates not only the geniohyoid muscle, but also in part the sternohyoideus, abdominohyoideus, and other trunk muscles. In the caecilians, in which it is derived from the first and second spinal and sometimes from the occipital as well, it innervates not only the geniohyoideus and hypoglossus, but also parts of the thoracicohyoideus and abdominohyoideus and other trunk muscles.

In general the hypobranchial nerve and musculature of selachians corresponds to the hypoglossal nerve and musculature of amphibians. There would seem little difficulty in deriving the amphibian hypoglossal from the selachian hypobranchial.

SYMPATHETIC GANGLIA CONNECTED WITH CRANIAL NERVES

The writers frankly disclaim any attempt at a description of the sympathetic nervous system in *Squalus*. Rather, there follow notes on the occurrence of certain ganglia, observed somewhat incidentally during the preparation of the main body of this research. Some of these ganglia have been seen and mentioned by previous writers, but as far as the writers know there has been no comprehensive treatment of the subject, as far as it concerns the selachians. These fragmentary observations are about all that could be made upon the material available. A special research upon material prepared especially for the investigation of sympathetic nervous tissue in the elasmobranch fishes should yield valuable results. The results of somewhat desultory

observations are here recorded in the hope that they may serve as a stimulus to more fruitful research.

The descriptions fall under two heads: 1. The ciliary sympathetic plexus; 2. The sympathetic ganglia upon the branchial nerves.

1. The ciliary sympathetic plexus

An extensive and confusing literature has developed around the subject of the ciliary ganglion. A misinterpretation of developmental conditions in the selachians has been responsible for much of the confusion. It is probable that some of the uncertainty is also due to the double or even multiple character of the ganglion in some selachians.

For recent reviews and discussion of the literature upon this subject the reader is referred to papers by Carpenter ('06) and by Neal ('14).

Keeping in mind the fact that in many selachians the ciliary ganglion because of its multiple character is in such minute parts as to escape ordinary notice in dissections, we see that Stannius ('49) has described with surprising accuracy the ciliary nerves and their relations to the oculomotor and trigeminal nerves. In the plagiostomes, according to him, there are usually two to four ciliary branches (long ciliary) arising from the ramus ophthalmicus profundus V (nerve or ganglion). Two of these enter the eyeball between the insertions of the rectus externus (lateralis) and rectus superior (dorsalis) muscles, and another, single or double, enters dorsal to the insertion of the rectus internus. A ciliary ganglion is lacking (in the opinion of Stannius) together with anastomoses between the ciliary branches of the oculomotorius and trigeminus. A ciliary branch of the oculomotorius arises from the ventral branch of the latter and accompanies a small artery to the eyeball, entering with it at a point between the insertion of the rectus internus and rectus inferior (ventralis). If Stannius had not overlooked the ciliary ganglia, his descriptions would fit very closely into the actual relations as found in *Squalus*. With the exception of the ciliary nerves, Stannius finds no definite sympathetic in the head of plagios-

tomes, but believes that the sympathetic elements are in these forms bound up with the cerebral nerves, going directly to their destinations without formation of special sympathetic trunks.

Schwalbe ('79) made an extensive study of the ciliary ganglion in the elasmobranchs. In *Scyllium catulus* he found three ciliary ganglia, one where the oculomotorius divides for the rectus inferior and obliquus inferior muscles, a second double in character situated approximately where the nerve crosses the ophthalmic artery, and a third found farther peripherally where the nerve breaks up in the inferior oblique muscle. In *Mustelus* he finds two ganglia, one where the branch to the rectus inferior is given off and the other farther peripherally on the branch to the obliquus inferior. In *Mustelus* a ciliary nerve from the ramus ophthalmicus profundus V runs to the eyeball, so close to the oculomotor nerve as to appear to be a branch of it. At about the same plane the oculomotorius sends a ciliary branch to the eyeball. In *Chimaera* he finds one ciliary ganglion, corresponding to the second one in *Mustelus*. He recognizes three classes of ciliary nerves: 1. motor, from the oculomotorius; 2. sensory, ciliares longi, from the ramus ophthalmicus profundus V; 3. vascular, ciliares breves, from the ciliary ganglion.

Ewart ('89) finds no ciliary ganglion in *Laemargus*, but one or two ciliary branches of the oculomotorius which, after joining ciliary branches of the ophthalmicus profundus, enter the eyeball. He states ('90) that occasionally two well-developed ciliary ganglia occur in *Acanthias*, connected usually with the inferior branch of the oculomotorius. In some instances ganglion cells have wandered along the ciliary nerves toward the eyeball. He finds no ganglion cells in the trunk of the third nerve or its branches. The ciliary ganglion has in all cases at least two roots, one from the oculomotorius and one or two from the ophthalmicus profundus. In addition to the ciliary nerves from the ciliary ganglion, there are ciliary nerves from both nerve and ganglion of the ophthalmicus profundus.

Hoffmann ('99) recognizes two ciliary ganglia in 48- to 50-mm. embryos of *Acanthias*, one situated approximately where the ophthalmic artery is crossed by the third nerve, the second

occurs laterally on the upper surface of the oculomotorius opposite the origin of the branch for the rectus internus. The second is connected directly with the ophthalmic ganglion through a nerve. The relation of the first ciliary ganglion to the ganglion of the ophthalmicus profundus is not evident. Gast ('09) figures in *Scyllium catulus* embryos of 39-mm. length at least five ciliary ganglia, the three most important of which he terms: 1) rectus ganglion, situated near the division of the third nerve into its dorsal and ventral branches; 2) ciliary ganglion, near where the branch to the rectus inferior is given off; 3) distal ganglion, apparently on the branch to the obliquus inferior. In *Mustelus laevis* of 22 mm. he finds three ciliary ganglia, rectus ganglion, distal ganglion, and ganglion on the 'infundibular nerve.' Allis ('01) recognizes a single ciliary ganglion in *Mustelus laevis*, which is connected with the ophthalmicus profundus by two branches (*radix longa*) and with the oculomotorius by a single branch (*radix brevis*).¹

In pointing out the fact that the so-called ciliary ganglion of many writers is the ganglion of the ophthalmicus profundus, Beard ('87) cleared up much of the prevalent confusion. The ganglion of the *ramus ophthalmicus profundus* he terms the mesocephalic ganglion, reserving the name ciliary for the ganglion intimately associated with the oculomotorius, which Schwalbe called the ganglion oculomotorii. Van Wijhe ('82) distinguishes between ciliary and oculomotor ganglia in *Scyllium*, but applies the former name to the profundus ganglion. He finds a nerve arising from the oculomotor (ciliary) ganglion and accompanying the ophthalmic artery to the eyeball. Marshall and Spencer ('81) and Marshall ('81) evidently confuse the ciliary with the profundus ganglion in the early embryos of *Scyllium*, and regard the profundus nerve as a part of the third nerve in origin. They report that ganglion cells of the ciliary in the later stages of development are found "in small scattered patches at different parts of the nerve" ('81, p. 89). The intimate relations of the meso-

¹ At the 1912 meeting of the American Association of Anatomists Dr. H. D. Senior, in a paper on the eye-muscle nerves of *Squalus acanthias*, stated that no ciliary ganglion occurs in that species.

cephalic and ciliary ganglia were recognized by Beard and by Onodi ('01). The latter examined the structures in the orbit of many selachians and came to the conclusion that the oculomotor ganglion (ciliary) of Schwalbe is a definite isolated ganglion in some species, a loose network of cells and fibers in others, and in others still in a form intermediate between these two extremes. In *Galeus* he finds a loose network; in *Mustelus laevis* two ciliary ganglia. These two ganglia are connected with each other by two branches, and each sends a ciliary nerve anteriorly, and a branch posteriorly into the ophthalmic nerve. From the larger of the two ganglia two branches with an oculomotor branch supply the ventral oblique muscle. As recently as 1905 Klinkhardt designated the profundus ganglion in *Spinax* as ciliary.

Connected with the oculomotor nerve in *Squalus* are a number of ganglia, varying in size from a half-dozen cells to many in number. These ganglia are situated on the courses of small non-medullated nerves which as a whole form a plexus. It is not possible in every instance to distinguish the fiber tracts upon which the ganglia are situated, being when in a loose structure so little differentiated from connective tissue. The number and arrangement of the ganglia is subject to individual variation. Moreover, the ganglion cells often occur scattered along their courses instead of being compacted into definite ganglia. There can, however, be distinguished the following general plan of arrangement and number.

Nothing but densely medullated motor fibers are detected in the intracranial portion of the oculomotor nerve. No intracranial ganglion cells, such as Nicholls ('15) finds in *Scyllium*, are found on the third nerve in *Squalus* or *Mustelus*. As the oculomotorius enters the orbit it divides at once into dorsal anterior and ventral posterior branches. At the point where this forking takes place a small ganglion occurs (figs. 8 and 21, *gcil.1*). sometimes of only about a half-dozen cells, sometimes within the oculomotor nerve sheath, more often just outside. Regarding the fiber connections of this ganglion there is some uncertainty. Possibly fibers run anteriorly along the anterior division of the oculomotor nerve together with blood-vessels; apparently they

do. Fibers can be traced from the ganglion or its vicinity anteriorly into the main nerve as it passes through the foramen in the cranial wall (fig. 6). From ganglion 1 there runs ventrally a fiber tract along the lateral border of the posterior (ventral) division of the III nerve, into a ganglion situated on the anterolateral border of the latter (figs. 9, 16, 17, 21, and 31, *gcil.2*). As described in the account of the ramus ophthalmicus profundus V, the posterior ciliary nerve includes a non-medullated constituent that turns ventrally and posteriorly to join the ciliary plexus. This non-medullated nerve passes, as stated, ventrally and posteriorly between the rectus dorsalis and the rectus lateralis muscles and enters ganglion 2. In some instances there is a small ganglion imbedded in the rectus dorsalis muscle between ganglion 2 and the ramus ophthalmicus profundus. Farther ventrally on the mesial posterior border of the third nerve at the point where the ventral division separates into branches, one for the rectus ventralis and the other for the obliquus ventralis muscle, there occurs another ganglion (ganglion 3) (figs. 10, 15, 17, and 21, *gcil.3*). Fibrous connection between ganglion 2 and ganglion 3 is plainly discernible as a flat band between the third nerve and the rectus lateralis muscle (fig. 20, *cil.*), as it passes around to the posterior mesial border of the third nerve. In some instances ganglion 2 and ganglion 3 are almost confluent (fig. 17); in other instances there seems to be another ganglion (ganglion 3a), distinct from ganglion 3, and situated in the forking of the nerve (fig. 10).

Ganglion 1 seems to correspond to Gast's 'rectus' ganglion, though possibly the latter also includes ganglion 2. Gast's 'ciliary' ganglion seems to correspond to ganglion 3. Hoffmann's two ciliary ganglia in *Acanthias* are evidently ganglia 2 and 3.

Ventral to ganglion 3, in the region of the ophthalmic artery, the ciliary sympathetic cord divides into two branches. One of these passing laterally around the posterior border of the oculomotor branch which supplies the ventral rectus muscle and then between the two oculomotor divisions, follows the ophthalmic artery out to the eyeball. On the way a ganglion (ganglion 6) occurs from which a small nerve runs anteriorly along the ventral

border of the ventral rectus muscle. Farther laterally, near the eyeball, occurs another ganglion (ganglion 7) from which the nerve passes into the eyeball (figs. 5, 7, and 21, *gcil.6*, *gcil.7*). This nerve running from ganglion 3 out to the eyeball is what is usually termed the ciliaris brevis. The second branch ventral to ganglion 3 extends posteromesially along the same small artery, but toward the origin of the latter from the pseudobranchial artery. At the dorsomesial border of the base of the infraorbital trunk (*mx.V + buc.VII*) a ganglion (ganglion 4) is found on this second ciliary branch (figs. 16 and 21, *gcil.4*). From this ganglion the ciliary branch runs posteriorly along the ophthalmic artery to the junction of the latter with the pseudobranchial artery, and farther posteriorly finally joining a twig of the ramus palatinus VII (fig. 21). Gast shows 'vascular nerves' in *Scyllium* which possibly represent in part this palatine anastomosis in *Squalus*. His infundibular nerve with ganglion in *Mustelus* seems to be the same nerve. Two or three small ganglia (ganglion 5) in *Squalus* on the ventral border of the rectus ventralis muscle near the ophthalmic artery are closely related to ganglion 3 (fig. 15, *gcil.5*). The small nerve from ganglion 6 running anteriorly along the ventral surface of the rectus ventralis muscle was not found in all specimens. When present it passes into a double (or single) ganglion (ganglion 8) imbedded in the oculomotor nerve branch destined to the ventral oblique muscle (fig. 21, *gcil.8*). Farther anteriorly, near where the third nerve breaks up in the ventral oblique muscle, a few ganglion cells (ganglion 9) are found, presumably connected by fibers with ganglion 8. Schwalbe's three ciliary ganglia in *Scyllium* are evidently ganglia 3 (with 3a or 6), 8 (double) and 9. His two ganglia in *Mustelus* seem to correspond to ganglia 3 and 8.

The writers would not be understood as laying much stress upon homologies between the ciliary ganglia of different species. There is too much individual variability to insist upon homologies based merely upon the situation of ganglionic masses.

The ciliary plexus in *Squalus* thus consists fundamentally of ganglionic masses in the orbit, related by non-medullated fibrous tracts to three distinct nerves: oculomotorius, ramus ophthal-

micus profundus V, and ramus palatinus VII. The medullated fibers given off from the ramus ophthalmicus profundus for the innervation of the eyeball, and passing to their distribution through the branches tentatively designated by the writers as anterior and posterior ciliary nerves, are apparently the equivalent of the ciliares longi of the higher vertebrates. They have nothing to do with the ciliary ganglion. The non-medullated constituent of the posterior ciliary fibers is the equivalent of the radix longa of human anatomy. Just where the oculomotorius gives its limited contribution to the ciliary nerve is difficult to determine, probably in the region of ganglion 1. A radix brevis is not clearly in evidence. The nerve that runs to the eyeball from ganglion 3 is without doubt the short ciliary nerve.

In *Mustelus californicus* there occurs a small group of ganglion cells where the oculomotor branch for the rectus superior muscle separates from the main nerve, corresponding apparently to ganglion 1 in *Squalus*. The ramus ophthalmicus profundus gives off a posterior ciliary branch as in *Squalus*, a medullated component of which passes posterolaterally to enter the posterior wall of the eyeball by two terminal branches, and a non-medullated part passes into the ventral division of the oculomotorius, but not through a small ganglion (ganglion 2) as in *Squalus*. The non-medullated element passes with the third nerve around the posterior border of the rectus inferior muscle and with the nerve runs anteriorly. A small ganglion occurs on the non-medullated element shortly after turning in the anterior direction. From this ganglion there pass three ciliary branches. One of these runs posterolaterally along the ophthalmic artery to the eyeball, entering the latter in a few small branches, near the entrance of the artery. This branch bears a ganglion, having also a small ganglion near its origin. A second branch from the ganglion passes posteromesially with the ophthalmic artery to the junction of the latter with the pseudobranchial artery and along the latter posteriorly to a junction with a branch of the ramus palatinus VII. From the relation of these two ciliary branches to the ganglion it would seem that the latter represents in a general way ganglion 3 in *Squalus*. The small ganglion on the

branch running out to the eyeball is possibly ganglion 7; the small ganglion near the beginning of the second nerve seems to be ganglion 4 of *Squalus*. A short distance anterior to ganglion 3 there occurs a large ganglion on the ventral branch of the oculomotorius, the one usually termed the ciliary ganglion of *Mustelus*.

It seems in some respects to answer to ganglion 6 in *Squalus*. As in the latter, a nerve runs anteriorly from the ganglion connecting with small ganglia upon the branch of the third nerve which supplies the ventral oblique muscle. Two such small ganglia are found more anteriorly near where the oculomotorius branch enters the oblique muscle. These anterior ganglia possibly correspond to ganglia 8 and 9 in *Squalus*. As in *Squalus*, an anterior ciliary nerve runs from the ramus ophthalmicus profundus to the eyeball. The arrangement of the ciliary ganglia and nerves in *Mustelus* is seen to be fundamentally similar to the conditions in *Squalus*. Gast's 'rectus' ganglion in *Mustelus* embryos of 22 mm. seems to be ganglion 1. His 'distal' ganglion is probably the large ganglion (ganglion 6), and his ganglion upon the 'infundibular nerve' is probably ganglion 4. Schwalbe's two ganglia in *Mustelus* occur upon the ventral branch of the third nerve. One is evidently the large ciliary ganglion (ganglion 6 or 6 and 3) and the other probably ganglion 8 or 9. Allis shows only one large ciliary ganglion in *Mustelus*.

2. *Branchial sympathetic ganglia*

From Stannius down to the present the opinion seems to have prevailed that the sympathetic system is represented in the head of selachians solely by the ciliary ganglion and its connections. The common opinion has been that the sympathetic elements, except for the ciliary, are contained indistinguishably in the cranial nerve trunks.

The writers find definite sympathetic ganglia on all the post-trematic rami of the branchial nerves, including the ramus hyomandibularis VII. With the exception of the latter, the typical number of these ganglia seems to be four for each post-trematic ramus, two on the dorsal region of the nerve as it passes across

the epibranchial cartilage, and two on the ventral region along the ceratobranchial cartilage. This number is subject to variation. The ganglia vary greatly in size and distinctness. In most instances they are situated on the bases of small branches of the posttrematic ramus, these small branches almost always containing motor fibers, so that motor nerves seem to arise from the ganglia. Non-medullated fibers connect with the ganglia and form conspicuous components of the nerve branches connected with the ganglia. In some instances non-medullated fibers connect adjacent ganglia external to the post-trematic ramus, forming semblances of plexuses. The non-medullated fibers, when they occur in large enough numbers, are in marked contrast to the medullated (lightly) fibers of visceral sensory nature. But in general the sympathetic fibers cannot be traced far from their ganglia because of the diffuse arrangement. No sympathetic ganglia were found by the writers on the pretrematic and pharyngeal rami of the branchial nerves.

The only reference the writers have found mentioning sympathetic ganglia upon branchial nerves is one by Allis ('01), who finds on the hyomandibular trunk of *Mustelus* a ganglion of small cells, from which pass four small nerves into the second dorsal constrictor muscle. The writers find this ganglion in *Mustelus* as Allis has described it; also other small ganglia on the more ventral branches of the same nerve.

As the hyomandibular trunk of *Squalus* passes dorsolaterally at the anterior wall of the spiracle the visceral sensory fibers are seen collected at the dorsolateral border of the nerve, the motor element at the ventrolateral edge, the main portion of the trunk being lateral line. In the transition from the anterior wall of the spiracle over to the posterior wall some of the visceral sensory fibers of the nerve are seen to pass around on both sides to the region of the motor fibers. Slightly farther ventral to this appear the sympathetic ganglia on small motor branches given off to the second dorsal constrictor muscle. Upon some of these branches (five or six) there are small ganglionic masses, the cells of which are much smaller than the smallest cells of the ordinary cranial nerve ganglia. Non-medullated strands, external to the

nerve trunk, connect some of these ganglia. The most ventral of this group on the main hyomandibular trunk is sometimes much larger than the others (figs. 46 and 47, *gsy.*).

After the hyomandibularis has divided into its chief rami, there may be found on the ramus hyoideus three or more of these small sympathetic ganglia. As the ramus hyoideus turns mesially around the lateral border of the hyoid bar to pass between the first and second ventral constrictor muscles, a small branch is given off to the second ventral constrictor. At the base of this nerve branch is a ganglion. Farther mesially, just ventral to the lateral border of the second ventral constrictor, is another ganglion, this also near the branching of the nerve. A distinct non-medullated nerve runs from this ganglion into the ramus hyoideus, and another non-medullated strand, accompanied by one or two medullated fibers runs from the ganglion anterodorsally into the constrictor muscle. Farther mesially still another small ganglion is situated at a branching of the ramus hyoideus, sending a non-medullated twig into the muscle. Scattered ganglion cells also are found upon the smaller divisions of the ramus hyoideus (fig. 48).

On the posttrematic rami of the glossopharyngeus and the first three branchial nerves of the vagus the arrangement is essentially as stated previously: two small ganglia on the ramus dorsally and two ventrally. Figure 48 shows striking variations from this arrangement, but the statement above holds true of most specimens. The ganglia are sometimes little more than scattered cells. Invariably they occur on small branches of mixed constitution. In the fourth branchial nerve of the vagus there are no motor elements, and ganglia are wanting on those portions of the posttrematic ramus passing over the epibranchial and ceratobranchial cartilages. The single elongate ganglion upon the posttrematic ramus is situated at the ventral border of the anterior muscular wall of the esophagus. Its cells are larger and of very different appearance from the cells of the ganglia on the other branchial nerves (fig. 48).

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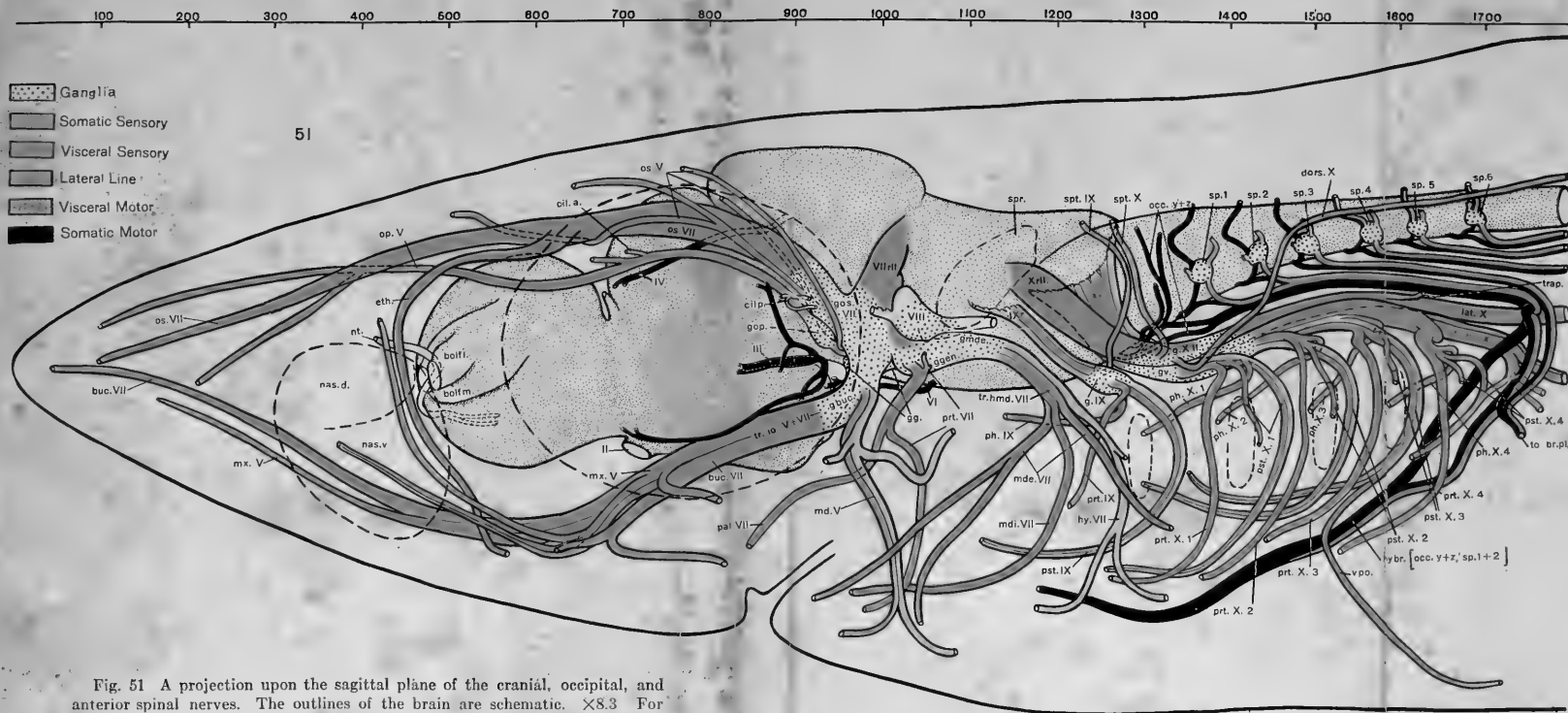
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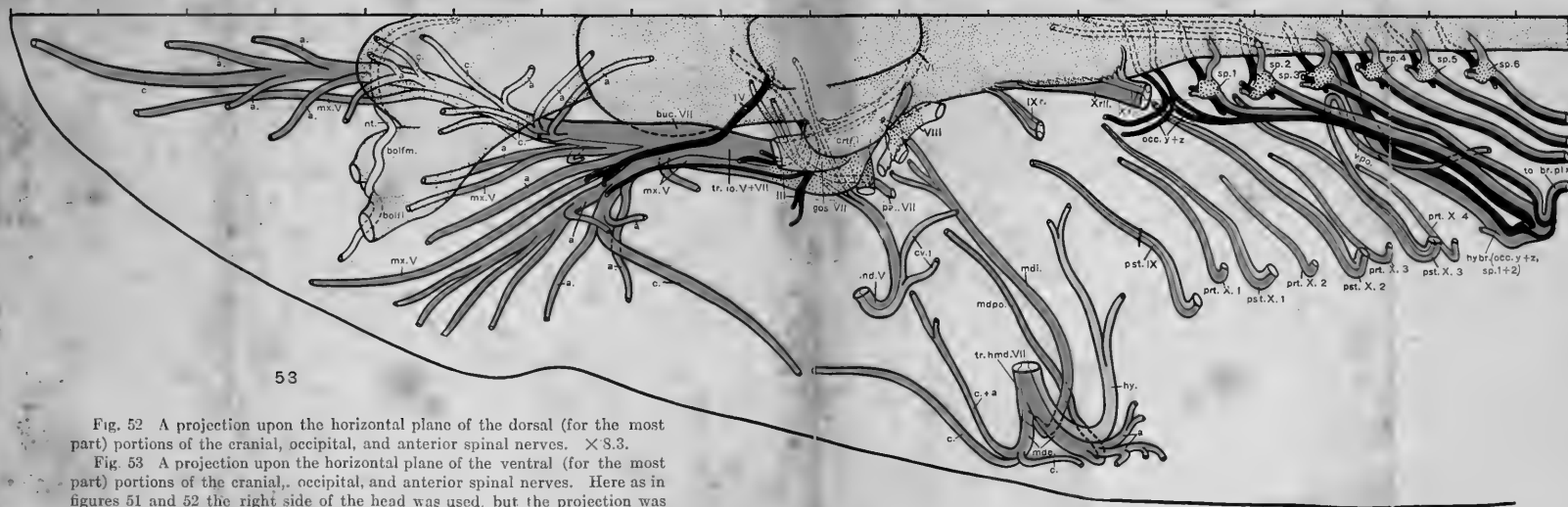
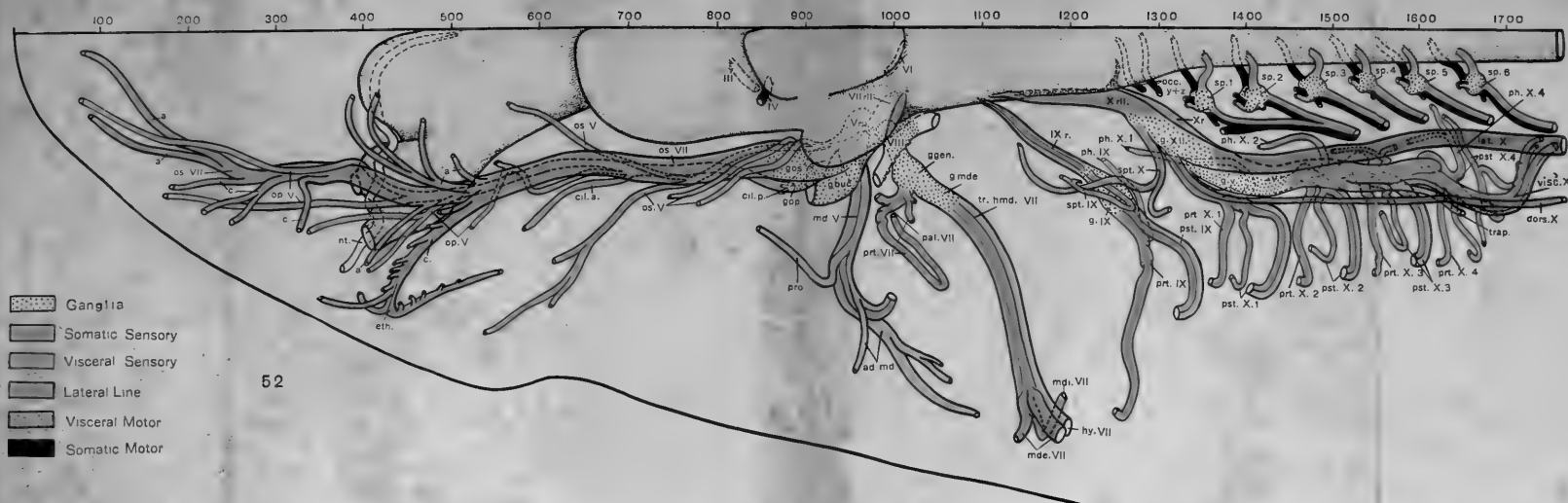


Fig. 52 A projection upon the horizontal plane of the dorsal (for the most part) portions of the cranial, occipital, and anterior spinal nerves. $\times 8.3$.

Fig. 53 A projection upon the horizontal plane of the ventral (for the most part) portions of the cranial, occipital, and anterior spinal nerves. Here as in figures 51 and 52 the right side of the head was used, but the projection was arranged to appear as of the left. $\times 8.3$. For abbreviations see pages 290-301.

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El sentido del olfato en los Ortópteros.

Poros olfatorios: El estudio comparativo de la disposición de los poros olfatorios de los Ortópteros se ha basado en el estudio de ambos sexos en veintiuna especies, pertenecientes a veinte géneros y representando seis familias. Se encontraron siempre poros olfatorios en las patas, antenas y estiletes anales; generalmente también en las alas (cuando existen), segmentos abdominales, cercos, cabeza y en todas las partes bucales y a veces también en los segmentos torácicos y oviscapto. En el primer segmento antenal generalmente existen unos pocos, pero hay siempre muchos de ellos en el segundo segmento. Los Mántidos y Fásmidos poseen el menor número de poros, ciertos acrídidos presentan el mayor número, mientras que las especies restantes tienen un número medio. Los poros en las seis mudas de los saltamontes aumentan gradualmente desde el 46 por ciento en la primera muda hasta 100 p.c., en la hembra adulta. Exteriormente estos órganos son oblongos generalmente, a veces casi presentan forma de hendidura, pero el tipo de poro en forma de ojo es el mas común. Interiormente cada uno de estos órganos posee una célula sensorial fusiforme cuyo extremo periférico se une con el orificio del poro en la quitina. Experimentos sobre las antenas: Se llevaron a cabo experimentos sobre los saltamontes y grillos para determinar si los llamados órganos olfatorios de las antenas reciben estímulos olfatorios. Puesto que se cortaron las antenas por encima de los poros olfatorios del primer y segundo segmento, parece probable, a juzgar por los tiempos de reacción obtenidos, que el resto de las antenas, que poseen los llamados poros olfatorios, no sirven como receptores olfatorios, en contra de lo supuesto por otros investigadores.

Translation by José F. Nonidez
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THE OLFATORY SENSE OF ORTHOPTERA

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NINETY-TWO FIGURES

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INTRODUCTION AND METHODS

The results herein recorded are a continuation of the writer's investigations concerning the olfactory pores of insects. Up to date, including the present results, these organs have been carefully studied in Hymenoptera, Coleoptera, Lepidoptera, Diptera,

and Orthoptera; also in one coleopterous larva and in thirty species of lepidopterous larvae. All of these orders, except Orthoptera, have complete metamorphoses, and consequently it was more convenient to study the olfactory pores in the adult forms than to use both immature and mature forms at the same time; but in regard to insects having incomplete metamorphoses, it is expedient to use all instars of at least one insect in the same study. For this reason and in order to determine what effect metamorphosis has on the olfactory pores, a careful study of the disposition of these organs in all six instars of a certain grasshopper has been made.

The two chief objects of the present investigation are: 1) to determine whether the olfactory pores are better adapted anatomically to receive olfactory stimuli than are the so-called olfactory organs on the antennae, and 2) to ascertain experimentally the effects on the olfactory sense when the so-called olfactory organs on the antennae are removed.

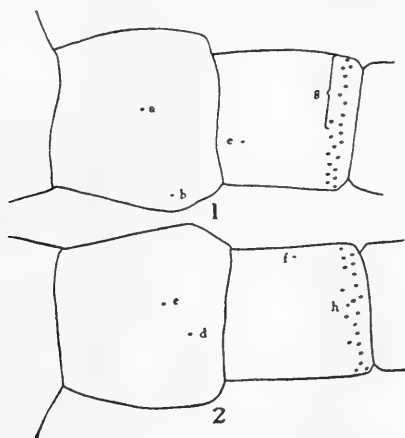
To obtain material for the study of the disposition of the olfactory pores, dried museum specimens were mostly used; these were obtained of Mr. A. N. Caudell, who also kindly identified all of the species used in this study. Fresh material was fixed in the modified Carnoy's fluid, and was embedded in celloidin and paraffin. The sections were cut 5μ in thickness, and were stained in Ehrlich's hematoxylin and eosin. All the drawings were made by the writer and all are original, except figures 90 to 92; these represent the so-called olfactory organs (pit pegs) and pegs on the antenna of a grasshopper (*Tryxalis nasuta* L.), and were copied from Röhler ('05). The drawings were made at the base of the microscope with the aid of a camera lucida.

MORPHOLOGY OF THE OLFACTORY PORES

Before making a study of the anatomy of the olfactory pores, the distribution and number of them were first investigated.

Disposition of pores in a grasshopper

From the eggs of *Melanoplus femur-rubrum* DeG., all six instars were reared, and since the fifth instar was most favorable in size and in condition of the integument for a critical study, the



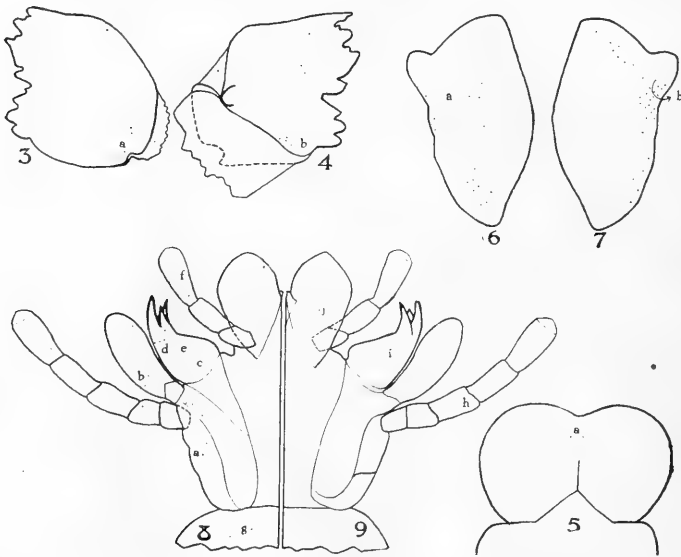
Figs. 1 and 2 Disposition of olfactory pores on the first and second antennal segments of female grasshopper (*Melanoplus femur-rubrum*, no. 18 in table 1) in fifth instar. Fig. 1, dorsal surface, and fig. 2, ventral surface of same segments; the inner edges of the antenna face one another. $\times 53$.

pores on a female of this stage were studied and drawn in detail as follows.

a. Pores on head. Pores were found on the head capsule and on all of the head appendages; on the former 7 scattered pores were observed, but on the head appendages they are more or less constant in position.

The first antennal segment bears 4 scattered pores, 2 being on the dorsal side and 2 on the ventral side (figs. 1 and 2, *a-d*); the second antennal segment bears 2 isolated pores (*e* and *f*), besides a group of 43 (*g* and *h*) which completely encircles the extreme distal end of the segment.

The mandible bears two groups of pores (figs. 3 and 4, *a* and *b*), one being on the outer side and the other on the inner side, and also several isolated pores. The labrum bears one group of 4 pores (fig. 5, *a*) on the ventral side. At the base of the hypopharynx there are two groups of pores, one of 32 (fig. 6, *a*) being on the left side and one of 27 pores (7, *b*) on the right side, besides



Figs. 3 to 9 Disposition of olfactory pores on mouth-parts of same grasshopper as mentioned in figures 1 and 2. Fig. 3, outer surface, and fig. 4, inner surface of same mandible; fig. 5, dorsal surface of labrum; fig. 6, outline of left side, and fig. 7, outline of right side of hypopharynx; fig. 8, ventral surface of one-half of maxilla and labium, and fig. 9, dorsal surface of same half of maxilla and labium. Figs. 3, 4, 5, 8, and 9, $\times 13$; figs. 6 and 7, $\times 21$.

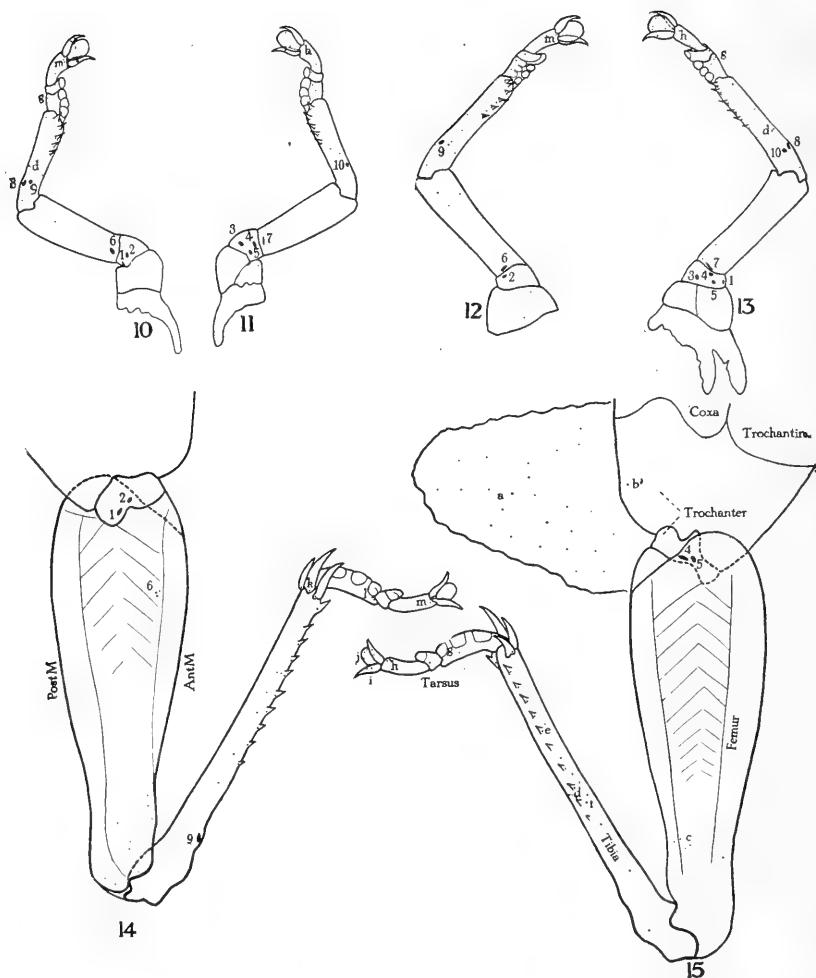
many isolated pores on either side about midway between the base and top of the appendage. Every portion of the maxilla and labium bears pores, 176 being found on the ventral side and 84 on the dorsal side; most of them are scattered, but four groups are present, two of these being on the ventral side of the lacinia (fig. 8, *c* and *d*), one on the dorsal side of the lacinia (fig. 9, *i*), and one on the dorsal surface of the third segment of the maxillary palpus (fig. 9, *h*). The scattered pores are represented in the

figures by widely separated dots, a few being present on each the palpifer (fig. 8, *a*) and the maxillary palpus, several on each the galea (fig. 8, *b*) and the lacinia (*e*), and a few on each the labial pulpus (*f*) and the ligula (fig. 9, *j*). The mentum bears 10 scattered pores (fig. 8, *g*).

b. Pores on thorax. Most of the pores found belonging to the thorax lie on the legs and wings and only a comparatively few are present on the thoracic segments. On each front and middle leg there are ten groups and several isolated pores, but on each hind leg there are only six groups besides several isolated pores (figs. 10 to 15). On each wing there is one group and several isolated pores (figs. 16 to 19).

The pores are located more definitely as follows: Groups nos. 1 to 5 (figs. 10 to 15) lie on the trochanter; nos. 6 and 7 on the femur; and nos. 8 to 10 on the tibia. All of these on the front and middle legs are constant in position, although slight variations may be observed owing to the degree in which the leg is rotated. On the smaller portion of the trochanter of the hind leg (figs. 14 and 15) there are only four groups, probably nos. 1, 2, 4, and 5. This part of the trochanter, partially hidden by the femur, is comparatively small and is found with difficulty. The groups on it are shifted in position and no. 1 has 8 pores instead of 3 as on the other legs; no. 2 has 9 pores instead of 10; no. 3 is absent, but on the other legs it has 9 pores; no. 4 has 5 pores instead of 8 or 10; no. 5 has 13 pores instead of 10. Groups nos. 6 and 7 on the front and middle legs lie at the proximal end of the femur, but at the same place on the femur of the hind leg there are no pores; no. 7 is wanting, but on the other legs it has 10 or 11 pores. No. 6 is either absent or has migrated down the femur (fig. 14) one-third the distance from the trochanter; however, at this position there are 3 pores, but no. 6 on the other legs has 13 pores. Groups nos. 8 to 10 lie near the proximal end of the tibia; nos. 8 and 10, having 8 and 5 pores, respectively, on the front and middle legs, are wanting on the hind leg, and no. 9 has 4 pores instead of 5.

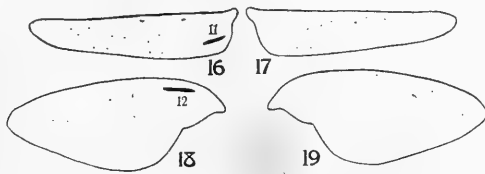
A few of the isolated pores are constant in number and position, while most of them are variable in disposition. The base of each



Figs. 10 to 15 Disposition of olfactory pores on legs of same grasshopper as mentioned in figures 1 and 2. Figs. 10 and 11, inner and outer surfaces, respectively, of front leg; figs. 12 and 13, inner and outer surfaces, respectively, of middle leg; figs. 14 and 15, inner and outer surfaces, respectively, of hind leg. The claws and pulvilli in figs. 10, 12, and 14 are shown from ventral view, and in figs. 11, 13, and 15 from dorsal view. *AntM* and *PostM* stand for anterior and posterior margins. $\times 6$.

pseudospine on the tibia (fig. 15, *e*) usually bears 1 pore, but occasionally 2 pores, and the base of each spine (fig. 14, *k*) always bears 1 pore. The following pairs of pores are always constant in position: 1 pair on each tibia (figs. 10, 13 and 15, *d*); 1 pair on the first tarsal segment (figs. 10 and 13, *g*) of the front and middle legs and 2 pairs on the same segment of the hind leg (figs. 14 and 15, *g* and *l*); 2 pairs on the third tarsal segment (figs. 10 to 15, *h* and *m*) of each leg, and 2 pairs on the dorsal surface of each claw and pulvillus (fig. 15, *i* and *j*).

The other isolated pores are represented in the figures by dots (fig. 15, *a*, *b*, and *c*); these are very variable in disposition and are much smaller than the other ones. All parts of the thoracic



Figs. 16 to 19 Disposition of olfactory pores on wings of same grasshopper as mentioned in figures 1 and 2. Fig. 16, dorsal surface, and fig. 17, ventral surface of front wing; fig. 18, dorsal surface, and fig. 19, ventral surface of hind wing. $\times 6$.

segments were not critically examined, but 39 minute pores (fig. 15, *a*) were counted on fragments of these segments.

Groups nos. 11 and 12 lie on the dorsal surface of the front and hind wing (figs. 16 and 18); no. 11 consisting of 15 pores and no. 12 of 19 pores. Several scattered pores are also present on both surfaces of each wing (figs. 16 to 19).

c. Pores on abdomen. In all, 45 pores were found on the abdominal segments. Roughly speaking, most of these lie in four rows extending the full length of the abdomen; two of these rows lie on the tergum and two on the sternum, the latter two near the midline, but the former two far from the midline. Each segment usually has at least 2 large pores and one or more minute ones, which may or may not be in the rows just mentioned. The ovipositor bears 53 scattered pores.

d. Pores on all six instars. A careful study of the pores on all six instars shows very little difference in regard to their distribution, but the total number of them on the different instars varies from 727 in the first instar to 1571 in the sixth instar (adult female). The first four instars are wingless while the wings of the fifth instar are comparatively small, as already shown in figures 16 to 19; nevertheless, the groups of pores on the adult wings are the same in number and position as are those of the fifth instar, but the adult wings bear only a few isolated pores. A few pores were found on the cerci of the first four instars, and many widely scattered ones lie on the ovipositors of the adult female and of the fifth instar (table 1). Now, since it is not expedient to tabulate the numbers of pores found on the various parts of the integument, they will be presented for the six instars in the following order: first, second, third, fourth, fifth, and six instar (♀ and ♂). Mandibles: 71-74-86-92-92- ♀ 86-♂108; maxillary palpi: 11-10-7-21-28- ♀ 56-♂24; galeae: 51-48-43-48-72- ♀ 56-♂62; laciniae: 52-48-48-56-104- ♀ 128-♂108; hypopharynx: 42-50-50-82-101- ♀ 110-♂108; labial palpi: 12-10-12-16-20- ♀ 44-♂20; ligula: 17-24-22-18-36- ♀ 26-♂38; labrum: 4-4-5-5-4- ♀ 4-♂4; mentum: 12-8-6-6-10- ♀ 10-♂10; total number of pores on mouth-parts: 272-276-279-344-467- ♀ 520-♂482; head capsule: 9-11-9-14-7- ♀ 5-♂7; antennae: 22-28-42-56-98- ♀ 106-♂110; abdominal segments: 54-45-54-50-45- ♀ 45-♂36; cerci: 3-4-3-5-0- ♀ 0-♂0; ovipositor: fifth instar ♀ 53-adult-♂55; legs: 367-429-449-485-660- ♀ 718-♂720; front wings: fifth instar 84- ♀ 84-♂86; hind wings: fifth instar 66- ♀ 38-♂40; total number of pores found on entire integument: 727-793-836-954-1480- ♀ 1571-♂1481. In obtaining these figures, the minutest and the most difficult pores to be found on the fifth instar have not been counted, nor have those found on the thoracic segments of the fifth instar been counted.

Disposition of pores in other Orthoptera

In making a comparative study of the disposition of the olfactory pores in Orthoptera, both sexes of twenty-one species,

belonging to twenty genera and representing the six families, have been examined. Since the pores on only one specimen for each sex in a species were counted, the total number of pores recorded cannot be a fair average.

a. Pores on head. Pores were found on all the head capsules examined, except on four locustids (table 1, nos. 37, 38, 41, and 42) and on the five crickets examined (nos. 43 to 47). The number varies from 0 to 40; the highest number being found in the American roach (no. 6).

The number of pores on the antennae vary from 16 (no. 24) to 124 (nos. 33 and 34). Pores are always present on the second antennal segments, and the numbers found on the first antennal segments are as follows: no. 1, 9; no. 2, 14; no. 3, 8; no. 4, 15; no. 5, 4; no. 6, 24; no. 7, 17; no. 8, 4; no. 9, 1; no. 16, 2; no. 17, 1; no. 18 (figs. 1 and 2), 8; no. 43, 2; no. 44, 4, and none on all the others. No pores were observed on the remaining antennal segments.

Relative to various portions of the mouth-parts of the adult specimens, the number of pores varies as follows: mandibles 8 (no. 9) to 110 (no. 2); maxillary palpi 0 (nos. 8, 10 to 13) to 77 (no. 6); galeae 0 (nos. 9 and 11) to 99 (no. 6); laciniae 2 (no. 9) to 166 (no. 34); hypopharynx 0 (nos. 10 to 13) to 216 (no. 39); labial palpi 0 (nos. 10 to 13) to 52 (no. 33); paraglossae (common to all except Acrididae) 0 (nos. 8, 9, 11, 46 and 47) to 89 (no. 4); glossae (common to all except Acrididae) 0 (nos. 8, 9, 11, 37 to 42, 44 to 47) to 18 (no. 5); ligula (common to only Acrididae) 0 (no. 15) to 56 (no. 24); labrum 0 (nos. 8, 9, 10, 12 and 13) to 31 (no. 39); mentum 0 (nos. 8 to 13, 37 and 38) to 35 (no. 43); and total number of pores on mouth-parts 30 (no. 11) to 605 (no. 43).

b. Pores on thorax. Relative to the legs and wings of the adult specimens, the number of pores varies as follows: legs 197 (no. 11) to 774 (no. 33); front wings 0 (nos. 23 and 24) to 134 (no. 33); and hind wings 0 (nos. 8, 9, 39 and 40) to 284 (no. 44). The front wings of nos. 23 and 24 are much reduced, about as long as the abdomen; they have grown together and are rigid like the elytra of beetles. The front wings of nos. 39 and 40 are much

reduced, and the hind wings are only rudimentary. The wings of the mole-cricket (no. 43) are very small, about one-half the length of the abdomen. The hind wings of the common cricket (nos. 44 and 45) are very small and the pores on them are minute. Only occasionally were a few pores observed on the thoracic segments.

c. Pores on abdomen. Relative to the pores on the abdomens of adult specimens, the number varies as follows: abdominal segments 0 (nos. 8 to 13, 35 to 47) to 178 (no. 1); cerci 0 (nos. 8 to 18, 24, 29, 32, 34, 35, 36, 38 to 42, 44 to 47) to 130 (no. 43); and ovipositor 0 (nos. 36, 42, 45 and 47) to 116 (no. 24). The male of *Blatta orientalis* (no. 4) and of *Periplaneta americana* (no. 6) has a pair of anal stylets which bear 42 pores, most of which lie on the dorsal surface. The cerci of these two species bear 51 pores, nearly all of which lie on the ventral surface at the extreme distal ends of the segments; however, in the grasshoppers and crickets the pores are widely scattered over the surface of the cerci. The pores on the cerci and anal stylets of nos. 4 and 6 have been added and then recorded under cerci.

The total number of pores found on the entire integument varies from 271 (no. 11) to 1616 (no. 33); the mantids and phasmids have the smallest number, certain acridids the largest number, while most of the remaining ones have a medium number.

d. Pores on first and last instars of croton-bug. Comparing the number of pores on the first instar (no. 3, recently hatched) with the number on the adult male (no. 1), we have the following figures: mandibles 31-68; maxillary palpi 5-14; galeae 15-74; laciniae 7-69; hypopharynx 8-40; labial palpi 7-13; paraglossae 4-45; glossae 0-9; labrum 7-15; mentum 7-6; total number on mouth-parts 91-353; head capsule 25-22; antennae 26-41; abdominal segments 126-178; cerci 11-82; legs 192-354; no front wings-47; no hind wings-11; and total number of pores found on entire integument 471-1088. Hence, the first instar has less than one-half the number of pores possessed by the adult; this was also found true for the grasshoppers.

e. Family, generic, specific, and sexual variations. Relative to these pores, the family variations may be small or large, depend-

ing on what families are compared; comparing the Acrididae, Locustidae, and Gryllidae with one another, the variations are small, but if these families are compared with the other three families or if Blattidae is compared with Mantidae and Phasmiidae, the variations are large. The chief variation pertaining to the genera, species, and sexes is in the number of pores present; however, the pores may occasionally differ a little in external structure. For example, those on the legs of the mole-cricket (no. 43) are almost slit-shaped, while in the other genera they are more or less eye-shaped. Twelve of the twenty males examined bear more pores than do their respective females, but, as a rule, there is not much sexual variation in the number of pores. For further details the reader is referred to table 1.

Structure of pores in a grasshopper

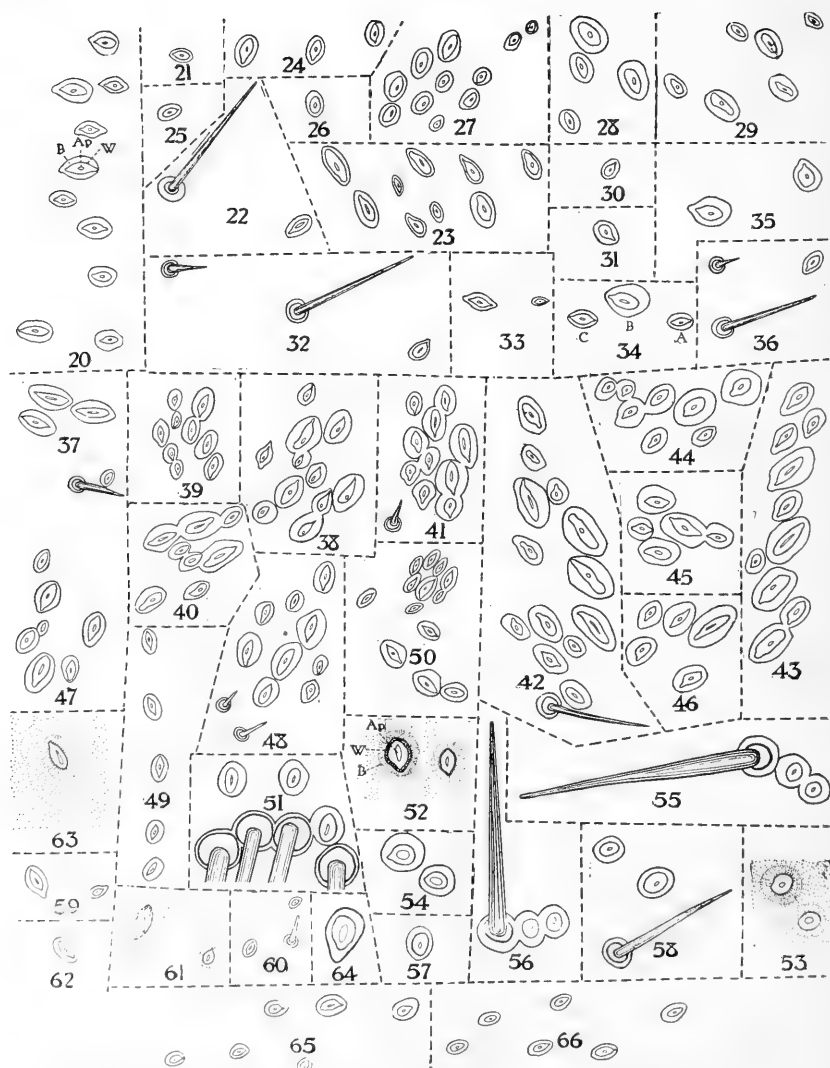
The preceding pages deal with the disposition of the olfactory pores, and now a discussion of their anatomy will be given.

a. External structure. When the superficial ends of the pores are examined under a high-power lens with a strong transmitted light, these organs appear as small bright spots, each of which is surrounded by darker chitin, the pore border (figs. 20 and 52, *B*), and by the pore wall (*W*). Sometimes the borders are scarcely discernible, as for example, those on the abdominal segments (fig. 33) and those of the smallest isolated pores on the legs (fig. 61); occasionally the borders and walls are very dark (figs. 15, *d*, and 52) and the wall is wide; a few of the pores have double borders (figs. 15, *f*, and 63), the outermost one being only a little darker than the surrounding chitin and the innermost one considerably darker; and occasionally the borders resemble some of those on coleopterous and lepidopterous larvae (McIndoo, '19) in that they have radial streaks (figs. 15, *g*, and 53). The pore wall may be round (fig. 55), oblong (fig. 51), but it is usually eye-shaped (figs. 20 and 22), and this is the first time for an eye-shaped type to be described; this type is common to all the Orthoptera examined; however, on the legs of the mole-cricket, the walls are almost slit-shaped, and in this respect somewhat resemble the lyriiform organs of spiders.

FAMILY	NUMBER AND NAME OF SPECIES	NUMBER OF PORES ON										TOTAL NUMBER OF PORES FOUND
		Head			Abdomen			Thorax		Hind wings		
		Mouth parts	Head capsule	Anten- nae	Abdom- inal seg- ments	Cerci	Ovipos- itor	Legs	Front wings (Teg- mina)			
Blattellidae.....	1. <i>Blattella germanica</i> ♂ adult.....	353	22	41	178	82		354	47	11	1088	
	2. <i>Blattella germanica</i> ♀ adult.....	397	19	38	143	57		347	39	17	1057	
	3. <i>Blattella germanica</i> 1st instar.....		91	25	26	11		192	†	†	471	
	4. <i>Blattella orientalis</i> ♂.....	391	32	60	27	94		473	53	11	1141	
	5. <i>Blattella orientalis</i> ♀.....	334	9	32	54	61		483	51	†	1024	
	6. <i>Periplaneta americana</i> ♂.....	510	40	84	6	93		324	38	10	1105	
	7. <i>Periplaneta americana</i> ♀.....	383	35	76	5	69		408	52	20	1048	
Mantidae.....	8. <i>Stagmomantis carolina</i> ♂.....	65	8	39	0	0		353	30	0	495	
	9. <i>Stagmomantis carolina</i> ♀.....	50	2	39	0	0		294	14	0	399	
Phasmidae.....	10. <i>Diaperomera femorata</i> ♂.....	58	8	32	0	0		201	†	†	299	
	11. <i>Diaperomera femorata</i> ♀.....	30	4	40	0	0		197	†	†	271	
	12. <i>Anisomorpha buprestoides</i> ♂.....	156	3	60	0	0		316	†	†	535	
	13. <i>Anisomorpha buprestoides</i> ♀.....	150	7	64	0	0		345	†	†	566	
	14. <i>Melanoplus atlantis</i> ♂.....	384	6	113	9	0		527	94	50	1183	
Acrididae.....	15. <i>Melanoplus atlantis</i> ♀.....	383	7	119	4	0	32	536	114	72	1267	
	16. <i>Melanoplus femur-rubrum</i> ♂ adult.....	482	7	110	36	0		720	86	40	1451	
	17. <i>Melanoplus femur-rubrum</i> ♀ adult.....	520	5	106	45	0	55	718	84	38	1571	
	18. <i>Melanoplus femur-rubrum</i> ♀ 5th instar.....	467	7	98	45	0	53	660	84	66	1480	
	19. <i>Melanoplus femur-rubrum</i> 4th instar.....	344	14	56	50	5		485	†	†	954	

Acrididae.....	20. <i>Melanoplus femur-rubrum</i> 3rd instar.....	279	9	42	54	3		449	†	†	836
	21. <i>Melanoplus femur-rubrum</i> 2nd instar.....	276	11	28	45	4		429	†	†	793
	22. <i>Melanoplus femur-rubrum</i> 1st instar.....	272	9	22	54	3		367	†	†	727
	23. <i>Tettigidea lateralis</i> ♂.....	278	2	24	43	21		325	0	61	754
	24. <i>Tettigidea lateralis</i> ♀.....	303	4	16	48	0	116	362	0	22	871
	25. <i>Eritettix simplex</i> ♂.....	241	5	70	30	16		562	32	30	986
	26. <i>Eritettix simplex</i> ♀.....	296	10	64	50	6	34	632	40	36	1108
	27. <i>Arphia sulphurea</i> ♂.....	349	5	87	34	24		582	32	24	1137
	28. <i>Arphia sulphurea</i> ♀.....	328	6	80	56	2	31	700	106	48	1357
	29. <i>Hippiseus tuberculatus</i> ♂.....	281	2	120	36	0		682	66	36	1223
Locustidae.....	30. <i>Hippiseus tuberculatus</i> ♀.....	300	4	110	6	12	22	624	82	30	1190
	31. <i>Dissosteira carolina</i> ♂.....	344	2	100	32	6		646	126	62	1318
	32. <i>Dissosteira carolina</i> ♀.....	455	9	120	22	0	101	734	108	60	1609
	33. <i>Schistocera americana</i> ♂.....	453	2	124	34	21		774	134	74	1616
	34. <i>Schistocera americana</i> ♀.....	441	3	124	26	0	20	640	120	12	1386
	35. <i>Orchelimum agile</i> ♂.....	410	4	88	0	0		591	132	96	1321
	36. <i>Orchelimum agile</i> ♀.....	446	3	100	0	0	0	608	33	134	1324
	37. <i>Microcentrum rhombifolium</i> ♂.....	272	0	65	0	41		493	88	48	1007
	38. <i>Microcentrum rhombifolium</i> ♀.....	292	0	63	0	0	11	500	80	52	998
	39. <i>Anabrus simplex</i> ♂.....	561	6	100	0	0		750	28	0	1445
Gryllidae.....	40. <i>Anabrus simplex</i> ♀.....	540	2	92	0	0	10	664	22	0	1330
	41. <i>Ceuthophilus uhleri</i> ♂.....	356	0	122	0	0		578	†	†	1056
	42. <i>Ceuthophilus uhleri</i> ♀.....	384	0	100	0	0		556	†	†	1040
	43. <i>Gryllotalpha hexadactyla</i>	605	0	46	0	130		252	60	8	1101
	44. <i>Gryllus pennsylvanicus</i> ♂.....	245	0	76	0	0		383	107	284	1095
	45. <i>Gryllus pennsylvanicus</i> ♀.....	295	0	74	0	0		348	128	194	1039
	46. <i>Oecanthus quadripunctatus</i> ♂.....	293	0	75	0	0		238	54	24	684
	47. <i>Oecanthus quadripunctatus</i> ♀.....	197	0	64	0	0		210	96	48	615
	Variation for adults.....	30-605	0-40	16-124	0-178	0-130	0-116	197-774	0-134	0-284	271-1616

† Wings wanting.



Figs. 20 to 66 External view of olfactory pores on same grasshopper as mentioned in figures 1 and 2. Fig. 20, 10 pores from *g* (fig. 1) on antenna; fig. 21, an isolated pore from *a* on antenna; fig. 22, one of the largest pores and medium-sized hair on head capsule; fig. 23, group *b* on mandible; fig. 24, group *h* on maxillary palpus; fig. 25, a pore from palpifer (fig. 8, *a*); fig. 26, a pore from galea (*b*); fig. 27, group *c* on lacinia; fig. 28, 4 pores of group *i* on lacinia; fig. 29, 6 pores from group *b* in hypopharynx; fig. 30, a pore from ligula (fig. 9, *j*); fig. 31, a pore from group *a* on labrum; fig. 32, a pore and 2 hairs near a spiracle on fourth abdominal seg-

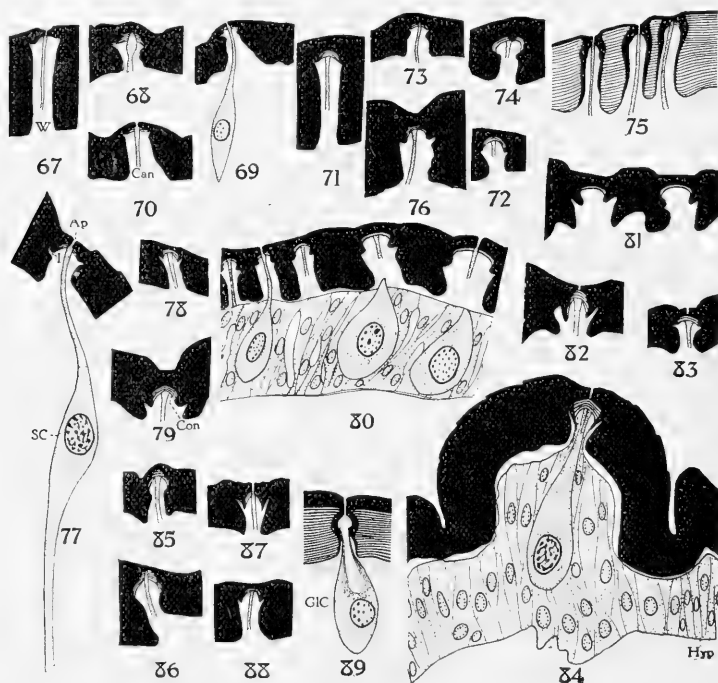
Inside the wall the chitin is lighter in color and near the center usually may be observed a round or oblong transparent spot, the aperture (figs. 20 and 52, *Ap*).

b. Internal structure. As in Lepidoptera and Diptera (McIndoo, '18), some of these pores belong to the dome-shaped type (figs. 68 to 70), because the chitin around the aperture is dome-shaped; most of them, however, belong to the hymenopterous type in that the chitin around the apertures is slightly depressed (figs. 77 and 82), and only occasionally is one (fig. 79) found approaching the coleopterous type, in which case the chitin is deeply depressed. Internally, these pores differ from all the others yet described by the presence of a cavity or indentation (fig. 77, *I*) encircling the base of the chitinous cone (fig. 79, *Con*). In cross-section this indentation resembles two horns which run from the pore canal (fig. 70, *Can*) outwardly into the pore wall (fig. 67, *W*); it is sometimes very shallow (fig. 71) and sometimes very deep (fig. 84), and since it is nearly always present in the grasshopper, we may regard these pores as constituting the orthopterous type.

The pore canal (fig. 70, *Can*) may be short (fig. 72) or long (fig. 67), but in the base of the pseudospines (figs. 15, *e*, and 84) on the tibia it passes only about one-half the distance through the integument, therefore, causing the pore aperture to be long.

ment; fig. 33, largest and smallest pore on last abdominal segment; fig. 34A, a pore from cercus (fourth instar); fig. 34B, a pore from cercus and fig. 34C, a pore from anal stylet of *Blatta orientalis*; fig. 35, 2 pores from ovipositor; fig. 36, one of largest pores, smallest and medium-sized hair from metathorax. Figs. 37 to 46, groups of pores on middle leg, and figs. 47 to 51, groups on hind leg (figs. 12 to 15); fig. 37, no. 1; fig. 38, no. 2; fig. 39, no. 3; fig. 40, no. 4; fig. 41, no. 5; fig. 42, no. 6; fig. 43, no. 7; fig. 44, no. 8; fig. 45, no. 9; fig. 46, no. 10; fig. 47, no. 1; fig. 48, no. 2; fig. 49, no. 4; fig. 50, no. 5; fig. 51, no. 6. Figs. 52 to 58, paired pores on legs; fig. 52, pair *d* on hind leg; fig. 53, pair *g* on middle leg; fig. 54, pair *e* on hind leg; fig. 55, pair *h* on hind leg; fig. 56, pair *m* on middle leg; fig. 57, 1 of pair *i* on claw of hind leg; fig. 58, pair *j* and a hair on pulvillus of hind leg. Fig. 59, largest and smallest pore on metathorax at *a* (fig. 15); fig. 60, largest and smallest pore at *b* on trochanter; fig. 61, largest and smallest pore at *c* on femur; fig. 62, pore on base of pseudospine at *e* on tibia; fig. 63, pore at *f* on tibia; fig. 64, pore on base of spine at *k* on tibia. Fig. 65, 6 of pores in group no. 11 on front wing; and fig. 66, 6 pores in group no. 12 on hind wing. *Ap*, pore aperture; *B*, pore border; *W*, pore wall. $\times 320$.

The chitin in sections usually did not take the stain, but remained a naturally dark color; however, in the labrum and at the tips of the labial palpi the outer stratum and pore walls are dark yellow (represented by black in figs. 75 and 89), while the re-



Figs. 67 to 89 Sections showing internal anatomy of olfactory pores of adult grasshoppers (*Melanoplus femur-rubrum*), soon after having molted the last time. Fig. 67, pore from mandible; fig. 68, pore from maxillary palpus; fig. 69, pore and sense cell from galea; fig. 70, pore from lacinia; fig. 71, pore from hypopharynx; fig. 72, pore from labial palpus; fig. 73, pore from ligula; fig. 74, pore from mentum; fig. 75, 3 pores from labrum; fig. 76, pore from head capsule; fig. 77, pore and sense cell from two sections through second antennal segment; fig. 78, pore from abdominal segment; fig. 79, pore from ovipositor; fig. 80, 5 pores and 3 sense cells from four consecutive sections through trochanter; fig. 81, 2 pores from femur; fig. 82, pore in group from tibia; fig. 83, isolated pore from tibia; fig. 84, pore and sense cell from base of pseudo-spine on tibia; fig. 85, pore from claw; fig. 86, pore from pulvillus; fig. 87, pore in group from front wing; fig. 88, isolated pore from hind wing, and fig. 89, gland pore and gland cell (*GLC*) from labrum. *Ap*, pore aperture; *Can*, pore canal; *Con*, chitinous cone; *Hyp*, hypodermis; *I*, indentation encircling chitinous cone; *SC*, sense cell, and *W*, pore wall. $\times 500$.

maining portions of the integument are semitransparent (represented by lines).

Many hypodermal gland pores (fig. 89) were observed on the ventral surface of the labrum; at first sight they may be mistaken for the 4 or 5 olfactory pores found on the dorsal surface of the labrum, but a careful study of them shows that they differ considerably in structure. The narrow aperture leads into the spherical reservoir, which connects with the gland cell (*GlC*); about one-half of the space in the peripheral end of this cell is occupied by the ampulla, in which the secretion apparently collects, and then this substance runs into the reservoir. The gland cell has only one pole, while the sense cell (fig. 77, *SC*) has two; and the olfactory organ has neither a reservoir, nor an ampulla. As far as known to the writer, these glands in the labrum of the grasshopper have never been described before, although the literature pertaining to hypodermal glands has not been consulted.

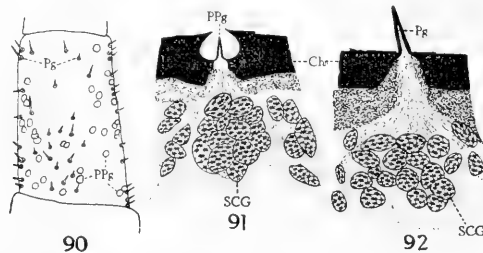
THE ANTENNAL ORGANS

Several investigators have studied the morphology of the antennal organs in Orthoptera, but since certain drawings of the acridid *Tryxalis nasuta* L., by Röhler ('05) best illustrate these organs, the following discussion will be taken only from his work.

The antenna of either a male or female of the preceding grasshopper consists of seventeen segments, which bear three types of sense organs as follows: The slender, strongly chitinized bristles are found on the first to eighth segments, but most of them lie on the third segment; the pegs (fig. 90, *Pg*) lie on all the segments, but most abundantly on the middle segments, and the so-called olfactory organs, pit pegs (*PPg*), lie on the third to seventeenth segments, but most abundantly on the tenth to fifteenth segments. The total average number of each type on one antenna is as follows: For males—77 bristles, 3738 pegs, and 1718 pit pegs, and for females—83 bristles, 2330 pegs, and 1362 pit pegs. From a superficial view, a pit peg resembles a small circle (fig. 90, *PPg*), but when viewed in section it is observed that a minute hair (fig. 91, *PPg*) with heavy walls arises from the bottom of the pit, and a peg (fig. 92, *Pg*) arises

from the outer surface of the chitin (*Ch*). Each of the three types of organs is supplied with a sense-cell group (*SCG*).

Röhler performed no experiments, but judging merely from the anatomy of these structures, he regards the bristles as tactile organs which probably regulate the movements of the antenna. He considers the larger or stronger pegs as tactile organs which protect the body from solid objects, and the smaller or weaker pegs are tactile organs which serve for the perception of wind drafts. After a long discussion, he concludes that the pit pegs are the olfactory receptors, and since the males have more pegs



Figs. 90 to 92 Structure of other antennal organs of a grasshopper (*Tryxalis nasuta*), copies from Röhler ('05); magnification not given. Fig. 90, one side of next to last antennal segment (16th), showing pegs (*Pg*) and pit pegs (*PPg*), the so-called olfactory organs; fig. 91, internal anatomy of pit peg, and fig. 92, same of peg. *Ch*, chitin, and *SCG*, sense cell group.

and pit pegs than have the females, he thinks that the former are better equipped for finding the latter than vice versa.

Röhler also found the sense bristles and pegs on the mouth-parts, but entirely overlooked the olfactory pores on the second antennal segments and on the mouth-parts.

According to Röhler (pp. 248 and 249), the following men studied the antennae of Orthoptera and report the following: In *Stenebothrus*, Kraepelin found pegs and pit pegs and in *Blatta* only olfactory hairs (probably pegs). In *Gryllidae*, *Locustidae*, and *Acerididae* vom Rath found both pegs and pit pegs, but in *Blatta* and *Periplaneta americana* only pegs. Graber concluded from his experiments that the antennae of *Blatta* function as olfactory organs. While searching for the Johnston's organ on the second antennal segments of *Locusta* and *Stenebothrus*,

Child saw the olfactory pores, described by the present writer, but he did not make a study of them; he soon decided, however, that they did not belong to the Johnston's type and did nothing further with them.

RESPONSES TO CHEMICAL STIMULI

In table 1 it is shown that an adult male or female (no. 16 or 17) of *Melanoplus femur-rubrum* has about 1500 olfactory pores, 108 of which lie on the second antennal segments; also, that an adult male or female (no. 44 or 45) of *Gryllus pennsylvanicus* has over 1000 pores, 75 of which lie on the second antennal segments. Using a pair of fine-pointed scissors, it was ascertained that the antennae could be cut off not nearer the head than through the third segments, therefore, five normal males and five females of each of the preceding species were selected for experimental purposes. Before mutilating them each one was placed in an experimental wire-screen case and was tested with the following sources of odors: chemically pure oils of peppermint, thyme, wintergreen and lemon, dried leaves of pennyroyal (odor very weak), and bran mash. The bran mash was made by using wheat bran, cheap molasses, lemons cut into fine pieces, and water; this mixture with arsenic added is the well-known poisoned bran mash, used for the control of grasshoppers, which are very fond of it either in the fields or in captivity, but crickets prefer bread or certain fruits to it.

Experiments with grasshoppers (Melanoplus femur-rubrum)

The following records include only the first responses and their reaction times:

a. *Unmutilated grasshoppers.* These individuals were apparently normal in all respects.

Oil of peppermint:

3 moved body slightly.

2 raised front legs.

2 moved backward slowly.

1 raised hind leg and turned around slowly.

1 turned around and worked mouth-parts.

1 turned to one side quickly.

Reaction time, 4 to 8 seconds; average, 5.8 seconds.

Oil of thyme:

5 raised front legs quickly and moved antennae.

3 moved away slowly.

1 arose quickly.

1 turned around slowly.

Reaction time, 3 to 15 seconds; average, 7 seconds.

Oil of wintergreen:

4 moved away slowly.

2 arose quickly.

2 moved backward slowly.

1 raised front leg.

1 moved body slightly.

Reaction time, 4 to 15 seconds; average, 7.3 seconds.

Oil of lemon:

3 moved backward quickly.

2 moved away slowly.

2 tried to get at source of odor through wire-screen.

1 raised front legs slowly.

1 arose slowly.

1 turned to one side quickly.

Reaction time, 3 to 10 seconds; average, 4.3 seconds.

Dried leaves of pennyroyal:

4 moved away slowly.

3 moved body slightly.

1 arose slowly.

1 moved front leg.

1 moved to one side.

Reaction time, 5 to 25 seconds; average, 14.8 seconds.

Bran mash (their food in captivity):

4 moved away slowly.

3 arose quickly.

1 moved to one side quickly.

1 moved body slightly.

1 tried to get at source of odor through wire-screen.

Reaction time, 5 to 20 seconds; average, 11 seconds.

The average reaction time of the males to the above six sources of odors is 7 seconds, and of the females 9.7 seconds, making a total average of 8.4 seconds for both sexes. The females were less responsive to all the odors, except to that of the bran mash, than were the males; but to bran mash each sex responded in 11 seconds.

b. Grasshoppers with antennae severed through third segments. These are the same insects used above; their antennae were cut off and twenty-four hours later were again tested with the same odors. As usual the females responded more slowly than did the males, and the total average reaction time of both sexes is 9 seconds, whereas it was 8.4 seconds before they were mutilated. They often tried to get at the bran mash and occasionally at the oil of lemon when these substances were held under the cases for a period of a minute or more. They were removed from the cases to a large cage where they ate bran mash, drank water, copulated, and lived just as long as did other individuals not mutilated.

Experiments with crickets (Gryllus pennsylvanicus)

The preceding experiments were repeated by using the common black cricket.

a. Unmutilated crickets. Since most of these ten insects failed to respond to the dried leaves of pennyroyal and to the bran mash, only the four essential oils were used as sources of odors. The average reaction time of the males to these odors is 10 seconds, and of the females 7.5 seconds, making a total average of 8.8 seconds.

b. Crickets with antennae severed through third segments. The antennae of the preceding crickets were cut off and two days later these insects were again tested with the same odors. The average reaction time of the males to the four odors is 7.5 seconds and of the females 12.9 seconds, making a total average of 10.2 seconds. Confined in battery jars containing moist sand, these mutilated crickets lived as long as others not mutilated; they ate bread and pieces of apples; the males chirped, and the females oviposited eggs in the sand as usual.

SUMMARY

In making a comparative study of the disposition of the olfactory pores in Orthoptera, both sexes of twenty-one species, belonging to twenty genera and representing the six families, have been examined; also, the pores on the first and last instars of the croton-bug (*Blattela germanica*) and on all six instars of the common grasshopper (*Melanoplus femur-rubrum*) have been carefully counted. Olfactory pores are more widely distributed in Orthoptera than in any other order yet studied. They were always found on the legs, antennae, and anal stylets; usually on the wings (if present), abdominal segments, cerci, head, and all the mouth-parts, and sometimes on the thoracic segments and ovipositor. Relative to the antennae, olfactory pores are present on only the first and second segments; occasionally a few lie on the first segment, but always many on the second segment. This is the first time that the writer has seen these organs on the antennae of adult insects, except several on the base of the antenna of the honey-bee and a few on the base of the antenna of a certain weevil; nevertheless, they are common to the antennae of all the larvae yet examined. The number of them on the wings is comparatively few, while the mouth-parts are abundantly supplied with pores.

The total number of pores found on the entire integument varies from 271 to 1616; the mantids and phasmids have the smallest number, certain acridids have the largest number, while most of the remaining species have a medium number. The newly hatched croton-bug has 44.5 per cent as many pores as has the adult female croton-bug; and comparing the total number of pores found on each of the six instars of the grasshopper (*Melanoplus femur-rubrum*), we have the following figures: first instar 46.3 per cent, second instar 50.5 per cent, third instar 53.2 per cent, fourth instar 60.7 per cent, fifth instar 94.2 per cent, adult male 94.3 per cent, and adult female 100 per cent.

In distribution and external structure, these olfactory pores resemble the lyriform organs of spiders more than do the same organs in any other order yet examined. They are generally ob-

long, sometimes almost slit-shaped, but the eye-shaped type is the most common. Some of the pore borders are radially striated; this is the first time for striated borders to be found in adult insects. The internal anatomy of these pores is similar to that of those in other orders, but there is one marked difference: in each of these there is an indentation or cavity which encircles the bottom of the chitinous cone.

Experiments were performed on grasshoppers and crickets to determine whether or not their antennae serve as olfactory receptors. The unmutilated insects were first tested to ascertain their reaction times to the oils of peppermint, thyme, wintergreen, and lemon and to the dried leaves of pennyroyal and to bran mash (their food in captivity). Each antenna was then severed through the third segment, and twenty-four hours later these mutilated insects were again tested with the above sources of odors. The average reaction time of the unmutilated grasshoppers is 8.4 seconds, and of them after being mutilated, 9 seconds; of the unmutilated crickets 8.8 seconds, and of the same crickets after being mutilated, 10.2 seconds. In other respects the mutilated individuals seemed normal and lived as long as others not mutilated. Since the antennae were cut off just distal to the olfactory pores on the first and second segments, it appears that the remainder of the antennal segments does not bear the olfactory organs as other investigators claim.

Compared with the so-called olfactory organs on the antennae of Orthoptera, the olfactory pores are better adapted anatomically to receive olfactory stimuli because the peripheral ends of their sense fibers come in direct contact with the external air, while those in the so-called olfactory organs on the antennae are covered with chitin.

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Resumen por el autor, Edward Horne Craigie,
Universidad de Toronto e Instituto Wistar.

Sobre la vascularización relativa de las diversas partes del
sistema nervioso central de la rata albina.

El autor ha llevado a cabo medidas anatómicas de la riqueza capilar en veintiuna regiones seleccionadas arbitrariamente en la médula espinal, médula oblonga y cerebelo de la rata albina. La parte mas pobre en capilares en la substancia gris es casi una vez y media mas rica que las zonas mejor dotadas de las regiones de substancia blanca estudiadas (fascículo longitudinal dorsal), mientras que este último presenta mas del doble de capilares que el fascículo cuneado. Los centros de la substancia gris pueden dividirse claramente en dos grupos: el de los núcleos motores y el de los centros sensorios y de correlación; estos últimos son los mas ricos en capilares. Aunque no existe una transición brusca entre estos grupos no existe tampoco una transición gradual, excepto en casos individuales. La única excepción se encuentra en el caso de la substancia gelatinosa. De todos los centros estudiados el mas rico es el núcleo coclear dorsal, que presenta una vascularización que excede en mas de la mitad a la de las astas ventrales (el centro motor mas vascularizado); en mas de dos veces y media a la de la substancia gelatinosa de Rolando (la región mas pobre de la substancia gris), siendo así mismo ocho veces mas rico en vasos sanguíneos que el fascículo cuneado. Existe muy poca diferencia entre ambos sexos. La interpretación de la significación funcional de los datos apuntados depende de problemas no resueltos sobre la naturaleza del proceso nervioso y su relación con el metabolismo.

Translation by José F. Nonidez
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ON THE RELATIVE VASCULARITY OF VARIOUS PARTS OF THE CENTRAL NERVOUS SYSTEM OF THE ALBINO RAT

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FIVE FIGURES AND FOUR CHARTS

The blood supply of the brain has been the object of study on the part of many anatomists from the time of Galen to the present day and the relations of the principal vessels have long been well known. The difference in vascularity between the gray and white matter in the brain and spinal cord has also been discussed by numerous authors. So far as the present writer has been able to discover, however, there does not seem to have been any attempt hitherto to make an exact quantitative comparison of the vascularity of the various structures in the central nervous system.

Ekker ('53)¹ made some general comparisons of capillary richness in various parts of the brain, but although he records some measurements of the diameter of vessels, nothing of importance is noted. According to him, the portion of the brain which is most richly supplied is the corpus striatum. The earlier work on this aspect of the subject was not very important. Guyot, in 1825, succeeded in isolating the vessels of the cerebral substance with forceps and a stream of water, and reached the conclusion that the white matter had no blood supply. Upon this observation he based the opinion that the white matter has no active function, but is entirely passive. Various writers

¹ The writer is indebted for information regarding this paper to Dr. C. Judson Herrick, who kindly reviewed it for him in the Surgeon General's Library at Washington. Later the writer had the privilege of examining a copy himself in the library of the College of Physicians of Philadelphia.

following Ekker (including Luschka, Henle, Frey) reproduced his account, but added nothing (according to Duret). Gerlach soon afterward made some similar observations, but without extending the knowledge of the subject.

The vessels on the surface of the brain and the main branches which enter its substance were well described in the eighteenth century by Haller, Willis, Vicq d'Azyr, and others, and their observations were extended and made more exact by many later workers.

Heubner ('72, '74) was "the first to investigate methodically the distribution of the different branches of the cerebral arteries" (Beever). He divided the arterial supply of the hemispheres into basal and cortical, the vessels of the former group being all 'end arteries,' without anastomotic intercommunications, while those of the latter group anastomose freely in the pia mater. Cohnheim ('72) concluded that there were a few anastomoses between the arteries near the circle of Willis, but that the arteries to the brain were practically 'end arteries' in the strict sense, and that anastomoses within the brain substance were insignificant when present at all.

About the same time, Duret ('73, '74) published an extensive study of the vascular supply of the brain. He found the cells of the bulbar nuclei to be surrounded by a very fine capillary net, while the mesh in the white tracts was large. In the cerebral cortex the outer 0.1 mm. contains large quadrangular meshes parallel to the surface, forming fine anastomoses between the arteries which penetrate the convolutions. The next 2 mm. is filled with rather fine polygonal capillary meshes, formed chiefly by collateral and terminal branches of the cortical arteries. The inner 1 mm. has a transitional network, with larger meshes, but much less elongate than those of the white matter, into which they pass. In the white matter the length of the meshes is three or four times the diameter of those in the gray matter, and they run parallel to the principal bundles, which they seem to surround. Throughout the central nervous system the cellular regions are more highly vascular than the rest. Duret remarks upon the fact that there is a complete correspondence in the vasculari-

zation of the whole cerebrospinal axis. The arteries of the bulb are divisible into median and radicular, corresponding to the groups supplying the hemispheres, and the same holds true regarding the spinal cord. He gives a detailed account of the origin of the vessels supplying each region of the brain.

Krause ('76) finds that the capillary network in the human spinal cord, in addition to being much wider meshed in the white matter than in the gray, is widest in the anterior funiculi, closest (in the white matter) in the posterior funiculi, particularly in the fasciculus gracilis.

The excess of the capillary supply of the gray matter over that of the white matter is noted by all succeeding authors who refer to the capillaries at all (Rudanowsky, Adamkiewicz, Kadyi, Hoche, Sterzi, Cajal, etc.). Rudanowsky ('76) describes the capillary network in the gray matter as being so fine as to encircle a single cell in each mesh. Adamkiewicz ('81) disagrees with him on this point, but finds evidence of a delicate secondary net within the primary capillary meshes, which does surround single cells as Rudanowsky describes.

The vessels of the spinal cord have been subjected to a careful study by a number of later writers, who have described in detail their development, arrangement, and distribution (Ross, Adamkiewicz, Kadyi, Hoche, Sterzi, Hoskins, etc.). Adamkiewicz finds the capillaries in the gray matter of the human cord to be relatively large, the net being densest and the capillaries largest in the cell groups. The net is poorer in the dorsal horns than elsewhere in the gray matter, except where these horns are as large as the ventral ones, in which case the net is alike in both.

Kadyi ('89) observes that the density of the capillary net is not the same in all parts of the gray matter of the spinal cord. He speaks, moreover, of 'true capillaries' (*echte Capillaren*)—those vessels which are interpolated between the final arterial branches and the first venous tributaries—of which the extent in the cord is rather small, and 'precapillaries' (*Vorcapillaren*), arterial and venous,—those vessels which divide into twigs of a still lower order, but yet resemble the capillaries in their lumen and in the structure of their walls. Hoche ('99) also makes

this distinction. Kadyi's nomenclature for the vessels on the surface of the cord has been adopted by succeeding workers.

Hoche, whose study is comparative, agrees fairly well with his predecessors. He finds that the difference in the capillary supply of the gray and white matter is much less in the rabbit than in the dog, where the ratio is about 2 or 3 to 1.

The most extensive comparative and embryological investigation of the blood supply of the spinal cord is that of Sterzi ('04), who describes it in all groups of vertebrates, from the cyclostomes up, and shows how the vascularization improves as one passes up the series. In studying the horse, he notes that the capillaries of a single mesh in the gray matter twist greatly, and that they are smaller at the head of the ventral columns, where the mesh also is closer. The capillaries of the white matter he finds to be larger than those of the gray matter, with which they communicate.

There is a good description of the vessels in the medulla oblongata due to Adamkiewicz ('90), while those of the midbrain have been studied by Alezais et D'Astros ('92) and by Shimamura ('94).

The arteries on the surface of the brain have been described repeatedly, the paper of Hofmann ('00) being of particular interest from the comparative standpoint. The comparative anatomy of the circle of Willis and its main branches in the mammalia has been well worked over by Tandler ('99, '02), to whose observations some interesting additions were made by Beddard ('04). These vessels were also the subject of an extensive phylogenetic, ontogenetic, and teratological study by De Vriese, which appeared soon afterward ('05).

The distribution of the vessels within the brain substance has also been described by several authors since those already mentioned. The most important of these are Beever ('09) and Stopford ('16). The former made a thorough and exact study of the source of the blood supply of each part of the forebrain in the human subject, while the latter made a similar investigation of the pons and medulla oblongata. The only paper of particular interest from the point of view of the present study

is perhaps that of Aby ('99). This investigator made a careful study of the arrangement and connections of the vessels in the cerebellum of the cat. He found the granular layer of the cerebellar cortex to be the most highly vascular, and observed that the layer of Purkinje cells was not different in its vascularization from the rest of the granular layer, as it might have been expected to be. He draws conclusions regarding the varying metabolic activity in the different layers based upon the assumption that, "at a given age, in a given organ, the relative number of blood capillaries in two regions is a certain index of the relative intensity of metabolic changes in those regions." This assumption may be compared with the observation of various authors that, "the richer any region is in nerve cells, the closer is the capillary network which supplies it" (Obersteiner, '90).

The only other point of interest in the literature which need be noted is the statement of Obersteiner ('90) that, "the corpus geniculatum laterale, corpus subthalamicum, and nuclei of the nerves are distinguished from the other gray masses by their richness in capillary vessels."

MATERIAL AND METHODS

The material used in this study consisted of the brains of nine albino rats and one hooded rat (no. 14), which were selected from among a great many preparations, their numbers being 12, 14, 16, 23, 24, 26, 31, 55, 56, 58. The animals were killed with illuminating gas and injected with carmin gelatin by means of a metal syringe, the cannula being inserted through the ventricle of the heart into the arch of the aorta, and the thoracic aorta being clamped. The brains were fixed in Bouin's fluid or in 10 per cent commercial formalin (nos. 23 and 26), imbedded in paraffin and cut, one sagittally (no. 12), the others transversely. The sections were 20 μ in thickness in all cases except no. 16, in which they were 15 μ . Only alternate sections were mounted except in no. 12. The material was stained with picric acid, either on the slides or in mass, the former being found best and being used in all the later work.

Injection with diluted India ink was tried, but gave less satisfactory results. As soon as injection was completed, the neck was ligatured and the whole head was cut off and immersed in chilled fixing fluid until the gelatin had time to set. The top of the skull was then opened and, if the gelatin was not yet firm, the head was returned to the fluid. Finally the brain was removed and left in the fluid for three and a half to five hours more. The earlier specimens were left longer, but the injection mass was found to be decolorized by the picric acid, so the time had to be cut down. For this reason several brains were fixed in 10 per cent formalin for five days, but this method was abandoned in favor of the brief treatment with Bouin, the findings of previous workers, as well as the observations of the writer, indicating that less distortion would thus be produced.

Bouin's fluid was used as a fixing agent for two reasons. In the first place, the strong formalin would give a good hardening of the gelatin; in the second place, Sugita ('17) has shown that this solution causes less change in volume than do others commonly employed.

After fixation the material was rinsed, dehydrated in the ordinary way with graded alcohols, and imbedded in paraffin.

To determine how much change this treatment produced in the brain, two specimens were carefully weighed and measured as soon as removed from the skull, and again after fixation and after dehydration. There was found to be practically no change in shape or size during fixation, though there was an increase in weight, as would be expected. After dehydration the changes were as follows:

	R 57, AGE 352 DAYS	R 58, AGE 390 DAYS
	<i>per cent</i>	<i>per cent</i>
Loss in weight.....	46.13	48.36
Loss in volume.....	36.61	37.58
Loss in area of sections.....	27.34	27.57

A considerable number of different stains were tried, but none were found to give a satisfactory contrast to the carmin gelatin except the picric acid. Although this gave a rather poor differentiation of the elements of the brain, it was found to be sufficient for the identification of the various parts and was used entirely.

In studying the sections, a square-ruled disc micrometer was used in a Leitz ocular no. 3. The objectives employed were Leitz nos. 3 and 7, and Bausch & Lomb 16 mm. and 4 mm. The total length of the pieces of capillaries enclosed by the square ruling (an area of 189μ square under Leitz objective no. 7) in each of ten sections was determined for each part studied in every brain, and the ten results were added together, giving the total length of the capillaries found in a block of tissue measuring $189 \times 189 \times 200$ c. μ . In the cases where the thickness of the sections was 15μ and where the Bausch & Lomb objective was used, the results were corrected to correspond with this.

There are several sources from which more or less serious experimental error may arise in this method of investigation. In the first place, there is the possibility of incomplete injection. If all brains which show evidence of incomplete injection are discarded, this source of possible error should not be serious when a number of brains are used.

While the fixation in Bouin's fluid causes relatively little distortion, the complete technique employed does produce a certain amount of shrinkage, as shown by Sugita and by the observations recorded above. No effort was made to correct for this, as it was considered that the relative vascularity of different regions would not be affected by it, provided that shrinkage is uniform throughout, as Sugita assumes to be the case. The material fixed in formalin gave somewhat lower results on the whole than the average values for the material fixed in Bouin's fluid. This is shown graphically in chart 1.

Any absolute determination of the vascularity by this method is, of course, impossible, and the investigation seeks merely to establish a set of ratios.

Hill ('96) apparently showed that the quantity of blood in the vessels of the brain when enclosed in the skull is practically

constant. The distribution of the blood, on the other hand, may vary in different physiological states, the greater part being in the arteries under certain conditions, in the veins and capillaries under others. The pressure may vary very widely, and is not necessarily the same as in other parts of the vascular system. The cerebral venous pressure and the pressure in the

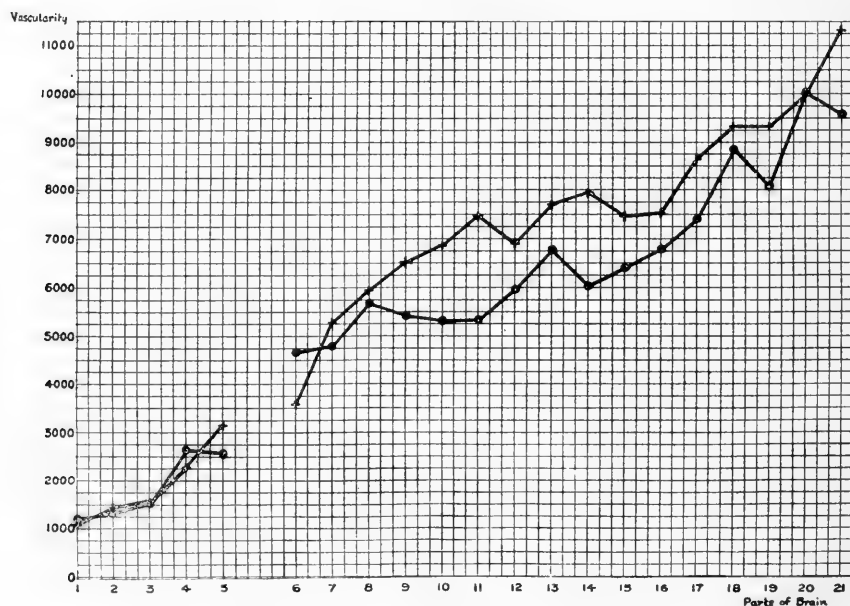


Chart 1 Graph showing the relation of the vascularities observed in material fixed in Bouin's fluid (Toronto animals only) and in material fixed in 10 per cent formalin. The break separates white and gray matter. Material fixed in Bouin, +; in formalin, •. Numbers of regions as in chart 2.

capillaries are always the same, however. A very careful reinvestigation in collaboration with Macleod ('01) merely confirmed Hill in his previous conclusions, in spite of the histological evidence of the presence of nerve fibers on intracranial vessels which had been brought forward by Huber ('99) and others. According to Corin,² the circle of Willis decreases the pressure of the blood before it reaches the brain.

² Cited by Stopford, quoting Poirer and Charpy's *Traite d'anatomie*.

Wiggers ('05, '07, '08) and Weber ('08), on the other hand, maintain that they have definite physiological evidence that the cerebral vessels are under nervous control, and in a recent paper (Orr and Rows, '18) a whole theory of the nature of certain lesions of the central nervous system is based upon the principle that the caliber of vessels entering from the pia mater is regulated by the sympathetic. This would further complicate the problem of injection, the effect of the injection mass and of the various experimental conditions upon the vasomotor mechanism being problematical. The earlier studies of Roy and Sherrington ('90), moreover, led them to conclude that "the chemical products of cerebral metabolism contained in the lymph which bathes the walls of the arterioles of the brain can cause variations of the caliber of the cerebral vessels," though no evidence of vasomotor control was obtained. If this is so, then it is at least possible that the injection mass may act directly on the walls of the vessels, causing them to change their diameter, and may even cause a different amount of change in the vessels of different regions. The most recent contribution to this problem is that of Weed and McKibben ('19), who seem to have good ground for their conclusion that "The *Monro-Kellie doctrine*³ then requires marked modification. . . . The cranial cavity is relatively fixed in volume and is completely filled by brain, cerebrospinal fluid, and blood; variations in any one of the three elements may occur, compensation being afforded by alteration in the volume of one or both of the remaining elements."

It might be thought that if the quantity of blood in the brain is constant when enclosed in the cranium, as maintained by Hill, an injection in this state would yield an absolute quantitative result. This does not appear to be the case, however. It is difficult to be quite sure that there is absolutely no leakage from the ligatured neck before the gelatin sets. Also the quantity of the injection mass remaining in the capillaries will vary with the varying distribution of the total amount of fluid within the cranium. The venous sinuses were always found to be largely filled with the mass, and it might have been expected that the

³ I.e., that the volume of blood in the brain is constant.

capillaries also would be so, since the capillary and the cerebral venous pressures are the same, according to Hill. The diameter of the capillaries in every case, however, was found to be from about $2\ \mu$ to about $4.5\ \mu$, a size which one would not consider at first sight to be natural. The diameter of the erythrocytes in the wild Norway rat has been determined by three observers to be 6.5 or $7\ \mu$ (Donaldson, '15). Some of the erythrocytes in the albino rats used in this study were measured (in the fresh condition) and were found to average about $6\ \mu$ in diameter. The average diameter of the capillaries in the human subject, where the corpuscles are just a little larger than in the rat, is stated to be about $12\ \mu$ (Cajal, '11; Halliburton, '14), so that one would expect those of the rat to measure at any rate 9 or $10\ \mu$. Sterzi ('04) finds the capillaries in the spinal cord of the horse to measure from 70 to $80\ \mu$. According to Koelliker, however, the finest capillaries in the human spinal cord are $5\ \mu$ in diameter, in the brain 4.5 , which, like my measurements, would be smaller than the erythrocytes. Moreover, a recent paper by Krogh ('19 a) makes it appear not improbable that the vessels in the preparations used for this study are about their natural size. Krogh finds that in living, resting muscle of the frog, the average diameter of the capillaries is about $4.5\ \mu$, while in the guinea-pig it is only $3.5\ \mu$, although the red corpuscles of the frog measure about $22 \times 15 \times 4\ \mu$ and those of the guinea-pig about $7.2\ \mu$ diameter by $2\ \mu$ in thickness. In passage, the corpuscles become greatly deformed and the walls of the capillaries yield somewhat where the corpuscles lie. Even in working muscle, where the vessels are found to expand greatly, the average diameter of the capillaries is still less than that of the corpuscles. It may be noted in passing that the same author, while demonstrating that a large number of the capillaries in resting muscle are completely closed, found that all the capillaries in the brain seemed to remain permanently open, so that there is not much danger of error from that source in the present case.

One factor in the apparent small size of the capillaries is the shrinkage of the gelatin injection mass. An attempt was made to measure this in some of the larger vessels, but it was found

to vary greatly, so that other factors are evidently concerned also. Where the shrinkage appeared greatest, the diameter of the mass was only about one-half of the diameter of the vessel in which it was contained. What changes the reagents may have produced in the caliber of the vessel itself could not, of course, be determined.

It was originally intended that the volume of the capillaries in a given volume of brain tissue should be measured, but it is evident from the above that measurements of the diameter of the vessels were of doubtful value. Moreover, there was found to be generally no very marked difference in the caliber of the capillaries in different regions, practically all sizes between the limits mentioned above occurring in each in similar numerical proportions, so that the ratio would be given just about as well by considering the lengths instead of the volumes.

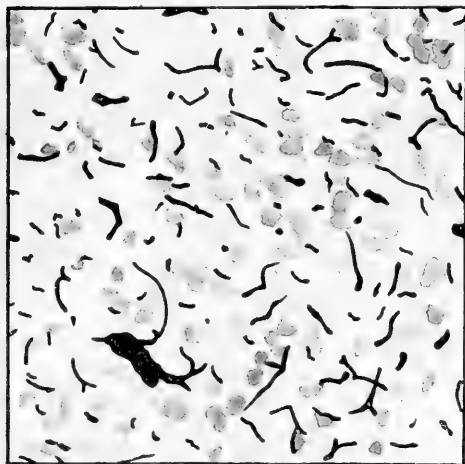
Finally, the possibility of error in measuring the vessels is considerable. It is no easy matter to make an accurate estimate of the length of a vessel which is twisting about in the thickness of the section and to make correct allowance for foreshortening, etc. When it is considered, however, that the result for each part represents the sum of from about 60 to about 350 measurements in each brain studied, this source of error may probably be neglected.

The particulars regarding the ten animals from which the results about to be detailed were obtained are as follows: nos. 12 and 16 were female albinos and no. 14 was a female hooded rat. These animals were obtained from a local dealer in Toronto and seemed to be in good condition. Nos. 23 and 24 were male albinos and no. 26 was a female albino. These were procured from the stock of the Department of Pathology of the University of Toronto. They were badly infested with lice and did not seem very healthy. All these animals were adults, not senescent, but of unknown age. They were not weighed or measured. Nos. 31, 55, 56, and 58 were albinos from the standard colony of The Wistar Institute, and full data concerning them were recorded.

RAT	SEX	AGE	WEIGHT	BODY LENGTH	TAIL LENGTH
		<i>days</i>	<i>grams</i>	<i>mm.</i>	<i>mm.</i>
31	♂	390	228.4	200	158
55	♀	406	159.2	185	177
56	♀	406	197.4	198	169
58	♂	390	234.8	210	172

OBSERVATIONS

The thickness of the sections was such that the complete capillary mesh did not show, the vessels appearing as short pieces, which were much more easily measured than they would have been if they had formed complete meshes within the field. That this appearance was due only to the thinness of the sections may be seen by comparing figures 1, 2, and 3, which represent parts of sections 20 μ thick, with figure 4, which represents part of a 50 μ section and figure 5, which is from one considerably thicker. The complete spongy mesh is not illustrated in the figures, as it was found impracticable to project satisfactorily upon one plane the complex system of capillaries seen in a section thick enough to show this, the course of the vessels being much contorted. Enough is represented, however, to indicate that such a condition actually exists, and one complete mesh appears in figure 5. The figures 1 to 3 were drawn with the aid of a Leitz-Edinger projecting microscope, while an ordinary microscope was used for projection in the preparation of figure 4 and figure 5. The illustrations show clearly the evident difference in vascular richness of the regions represented. In figure 1 and figure 2 the more conspicuous cell bodies are indicated in gray to show their relation to the vessels. Nowhere were the meshes of the capillary net small enough to surround single cells, as described by Rudanowsky, nor were there any traces of a delicate secondary network filling the primary meshes (Adamkiewicz, loc. cit.). The smallest meshes noticed were about 80 μ in diameter, but no study of this matter was made, as the contorted course of the vessels caused such measurements to be practically useless, besides preventing a complete loop from lying in one section unless this was very thick.



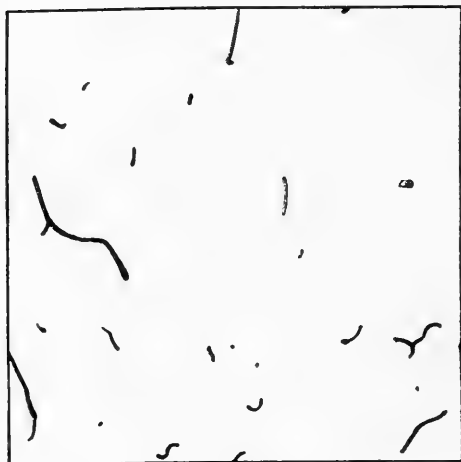
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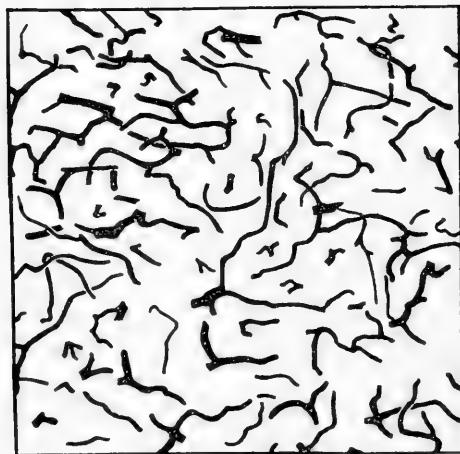
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Fig. 1 Part of a section of the right chief vestibular nucleus. $\times 160$. Thickness, $20\ \mu$. (R.23.)

Fig. 2 Part of a section of the right hypoglossal nucleus. $\times 160$. Thickness, $20\ \mu$. (R.23.)



3



4

Fig. 3 Part of a section of the right ventral funiculus of the spinal cord. $\times 160$. Thickness, $20\ \mu$. (R.23.)

Fig. 4 Part of a section of the right chief vestibular nucleus. $\times 160$. Thickness, $50\ \mu$. (R.43.)

With regard to the diameter of the vessels, while Adamkiewicz states that those in the gray matter of the spinal cord are relatively large, Koelliker ('96) and Sterzi (*loc. cit.*) find the capillaries in the white matter to be larger than those in the gray matter, while Hoche states that the white matter is supplied chiefly by 'Vorcapillaren,' the gray by 'echte Capillaren' in the dog. The last author found less difference in the rabbit, however. As indicated above, there was no marked distinction to be seen in the present case.

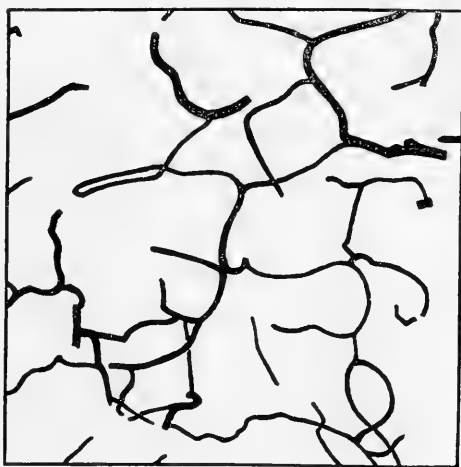


Fig. 5 Part of a thick section of the white matter of the cerebellum. $\times 160$. The drawing was made from a rather thick part of a wedge-shaped section, so that the exact thickness is unknown. (R.14.)

The linear measurements obtained are recorded in the accompanying table 1, the actual value for each part being given, along with its ratio to the respective results for the ventral funiculus and the ventral cornu of the spinal cord of the same animal. The various regions are arranged in the table in order of increasing richness of blood supply, as determined by averaging all the values for each. When this is done, two horizontal lines can be drawn, one separating the figures for the white matter from those for the gray, the other separating the results for the motor centers from those for the sensory and correlation centers. Thus,

TABLE 1
Showing linear measurements obtained and their ratios

	LOCALITY	R. 12			R. 14			R. 16			R. 23			R. 24		
		μ per 189 \times 200 c. μ	Ratio Vent. white	Ratio Vent. gray	μ per 189 \times 200 c. μ	Ratio Vent. white	Ratio Vent. gray	μ per 189 \times 200 c. μ	Ratio Vent. white	Ratio Vent. gray	μ per 189 \times 200 c. μ	Ratio Vent. white	Ratio Vent. gray	μ per 189 \times 200 c. μ	Ratio Vent. white	Ratio Vent. gray
		1	1.272	1.03				962	0.76	0.45	3,501	2.11	0.54	5,211	3.72	0.94
	Fasc. cuneatus ¹	2	1.237	1.00				1,258	1.00	0.80	6,009	3.62	0.92	5,589	3.99	1.00
	Ventral column.....	3	1.528	1.23				1,436	1.14	1.01	5,326	3.21	0.82	6,544	4.67	1.17
	Lateral column.....	4	2.134	1.72				2,139	1.70	0.92	6,912	4.17	1.06	5,699	4.07	1.02
	Pyramidal tract.....	5	2.785	2.25				3,964	3.15	1.00	6,429	3.94	1.00	5,573	3.98	1.00
	Fasc. long. dors.....															
		6	2.492	2.01	0.36			3,295	2.62	0.45	3,501	2.11	0.54	5,211	3.72	0.94
	Subst. gelat. Rolandi.....	7	4.556	3.68	0.66			5,879	4.67	0.80	6,009	3.62	0.92	5,589	3.99	1.00
	Nuc. mot. VII.....	8	5.158	4.16	0.75			7,430	5.91	1.01	5,326	3.21	0.82	6,544	4.67	1.17
	Nuc. XII.....	9	6.726	5.43	0.97			6,785	5.39	0.92	6,912	4.17	1.06	5,699	4.07	1.02
	Nuc. mot. V.....	10	6.922	5.59	1.00			7,372	5.86	1.00	6,429	3.94	1.00	5,573	3.98	1.00
	Ventral horn; cord.....															
		11	8.350	6.74	1.21			7,637	6.07	1.04	8,125	4.90	1.25	5,948	4.24	1.07
	Spinal V. nucleus.....	12	6.146	4.96	0.89			7,588	6.03	1.03	7,431	4.48	1.14	6,107	4.36	1.10
	Deiters' nucleus.....	13	6.621	5.35	0.96			7,966	6.33	1.08	7,829	4.72	1.20	7,275	5.12	1.31
	Molec. layer; cerebellum ²	14	8.000	6.46	1.16			7,824	6.22	1.06	7,521	4.54	1.15	6,162	4.40	1.06
	Dors. horn; cord.....	15	6.460	5.21	0.93			7,835	6.23	1.06	8,333	5.02	1.28	7,443	5.31	1.34
	Inferior olive.....	16	6.771	5.47	0.98			8,391	6.67	1.14	7,260	4.38	1.11	7,800	5.56	1.40
	Superior olive.....	17	8.371	6.76	1.21			8,686	6.90	1.18	9,771	5.86	1.44	7,418	5.29	1.33
	Chief sens. V nucleus.....	18	8.574	6.92	1.24			9,197	7.31	1.25	9,415	5.68	1.49	9,232	6.59	1.66
	Granule layer; cerebellum ²	19	8.870	7.16	1.28			9,186	7.30	1.25	10,544	6.36	1.61	7,603	5.42	1.36
	Nuc. dentatus.....	20	8.878	7.17	1.28			11,277	8.96	1.53	10,941	6.60	1.68	8,924	6.37	1.60
	Chief vestib. nucleus.....	21	9.945	8.03	1.44			9,495	7.56	1.29	13,285	8.01	2.04	10,158	7.24	1.82
	Dors. cochlear nucleus.....															

TABLE 1—Continued

	LOCALITY	R. 26				R. 31				R. 55				R. 56				R. 58				AVER- AGE	PROBABLE ERROR OF AVERAGE
		Ratio		Ratio Vent. gray	Ratio Vent. white	Ratio Vent. gray	Ratio Vent. white	Ratio Vent. gray	Ratio Vent. white	Ratio Vent. gray	Ratio Vent. white	Ratio Vent. gray	Ratio Vent. white	Ratio Vent. gray	Ratio Vent. white	Ratio Vent. gray	Ratio Vent. white	Ratio Vent. gray	Ratio Vent. white				
		μ per 189°X200 c. μ	μ per 189°X200 c. μ																				
		1 1,234	0.93		1,727	1.27		1,698	1.08		1,463	1.01		1,413	1.09		1,318	3.2					
	Fasc. cuneatus ¹ ...	2 1,331	1.00		1,365	1.00		1,569	1.00		1,450	1.00		1,502	1.00		1,413	2.1					
	Ventral column...	3 1,399	1.05		1,413	1.04		1,880	1.20		1,832	1.26		1,371	1.05		1,593	2.6					
	Lateral column .	4 2,699	2.03		2,323	1.70		2,947	1.83		2,852	1.97		2,488	1.91		2,501	2.5					
	Pyramidal tract	5 2,326	1.75		2,882	2.11		3,518	2.23		2,797	1.93		3,310	2.54		3,041	3.2					
	Fasc. long. dors..																						
	Subst. gelat. Ro-	6 4,008	3.07	0.82	4,261	3.12	0.79	5,598	3.56	0.78	3,124	2.16	0.44	4,957	3.81	0.75	4,159	5.4					
	landi.....	7 3,974	2.99	0.79	4,285	3.14	0.80	5,779	3.68	0.81	5,742	3.96	0.81	5,863	4.50	0.89	5,230	3.2					
	Nuc. mot. VII....	8 4,848	3.64	0.97	4,861	3.56	0.90	6,839	4.36	0.96	4,618	3.18	0.65	5,966	4.58	0.90	5,732	3.5					
	Nuc. XII.....	9 5,120	3.85	1.02	4,726	3.46	0.88	5,957	3.80	0.84	4,863	3.35	0.68	5,976	4.59	0.90	5,837	2.9					
	Nuc. mot. V.....																						
	Ventral horn;	10 5,005	3.76	1.00	5,374	3.94	1.00	7,132	4.54	1.00	7,123	4.91	1.00	6,612	5.08	1.00	6,430	2.7					
	cord.....																						
	Spinal V nucleus	11 4,711	3.54	0.94	6,016	4.41	1.12	6,790	4.33	0.95	5,551	3.83	0.78	6,962	5.35	1.05	6,592	3.8					
	Deiters' nucleus	12 5,811	4.37	1.16	6,279	4.60	1.17	7,854	5.02	1.10	6,381	4.40	0.90	6,803	5.23	1.03	6,677	2.9					
	Molecular layer;																						
	cerebellum ²	13 6,253	4.70	1.25	5,514	4.04	1.03	7,267	4.63	1.02	6,067	4.18	0.85	7,963	6.11	1.20	7,116	2.9					
	Dorsal horn;																						
	cord.....	14 6,124	4.60	1.22	6,171	4.52	1.15	7,698	4.91	1.08	6,582	4.54	0.92	7,512	5.77	1.14	7,203	2.6					

¹ All the measurements for parts in the spinal cord were made in the third cervical segment.² The measurements were made in the posteroventral region of the vermis (uvula).

TABLE 1—*Continued*

LOCALITY	R. 26			R. 31			R. 55			R. 65			R. 58			AVER- AGE	PROBABLE ERROR OF AVERAGE
	μ per 189 \times 200 c. μ	Ratio Vent. white	Ratio Vent. gray	μ per 189 \times 200 c. μ	Ratio Vent. white	Ratio Vent. gray	μ per 189 \times 200 c. μ	Ratio Vent. white	Ratio Vent. gray	μ per 189 \times 200 c. μ	Ratio Vent. white	Ratio Vent. gray	μ per 189 \times 200 c. μ	Ratio Vent. white	Ratio Vent. gray		
Inferior olive	5,334	4.01	1.07	6,625	4.85	1.23	9,342	5.95	1.31	8,700	6.00	1.22	9,535	7.32	1.44	7,693	3.6
Superior olive	5,743	4.32	1.15	6,707	4.92	1.25	10,457	6.60	1.45	9,534	6.58	1.34	9,682	7.44	1.46	8,008	4.0
Chief sens. V nu- cleus	7,365	5.53	1.47	6,748	4.94	1.26	8,148	5.19	1.14	7,723	5.33	1.08	8,674	6.66	1.31	8,072	2.3
Granule layer; cerebellum ¹	8,542	6.42	1.71	6,688	4.90	1.24	8,668	5.52	1.22	7,876	5.43	1.11	9,215	7.08	1.40	8,762	2.3
Nuc. dentatus	8,596	6.46	1.72	8,097	5.93	1.51	9,988	6.37	1.40	9,591	6.61	1.35	9,636	7.40	1.46	9,089	2.7
Chief vestib. nu- cleus	11,131	8.36	2.22	7,682	5.63	1.43	10,288	6.55	1.44	8,706	6.00	1.22	10,677	8.20	1.62	9,742	2.8
Dors. cochlear nucleus	9,056	6.80	1.81	8,416	6.16	1.57	11,886	6.91	1.67	10,058	6.93	1.41	11,511	8.84	1.74	10,523	3.0

¹ The measurements were made in the posteroventral region of the vermis (uvula).

a mere glance at the table shows the striking fact that the sensory centers are more richly supplied with blood-vessels than are those of which the function is motor, except in the single case of the substantia gelatinosa Rolandi of the spinal cord.

Among the motor centers upon which observations were made, the poorest is the motor nucleus of the facial nerve, the richest the ventral cornu of the spinal cord. The richest of the sensory centers studied, on the other hand, is the dorsal cochlear nucleus, while after it come, respectively, the chief vestibular nucleus, the dentate nucleus, and the granular layer of the cerebellar cortex. It will be noticed that the last three mentioned are related to each other, and it may be remarked that these four richly vascularized regions are ones which are probably in more or less constant receipt of stimuli, and so may be assumed to be in almost continuous activity, which may decrease in intensity at times, but in all probability ceases more rarely than does the activity of many other centers. It is interesting to observe that even the molecular layer of the cerebellar cortex, though distinctly poorer than the granular layer, is more richly supplied with capillaries than the motor, and even than some of the sensory centers. Krause (*loc. cit.*) remarked that the inferior olive and the dentate nucleus are conspicuous in their respective regions on account of their richness in capillaries. The relative vascularity of the various regions studied is shown graphically in chart 2.

The present data correspond fairly well with the rather indefinite statement of Hoche (*loc. cit.*) regarding the relative vascularity of gray and white matter in the spinal cord. They do not, however, agree with his statement that part of the dorsal funiculus is the richest part of the white matter in the dog, nor with Krause's findings on the relative richness of the white columns in the human cord; the results of the present study indicating that in the rat the pyramidal tract and the lateral funiculus are richer than the ventral funiculus and the fasciculus cuneatus, which are about alike.

The pyramidal tract in the rat is situated in the ventral portion of the dorsal funiculus of the spinal cord, where it is rather sharply marked off from the rest of the white matter. It stains distinctly

by various methods on account of the fact that it is very incompletely myelinated in this animal, and that those myelin sheaths which are present are very thin (Ranson, '13, '14). This tract was found to stand out quite clearly, even with the simple picric-acid stain, so that it was easy to consider it by itself, which was fortunate, since the difference in vascularity between it and the neighboring fasciculus cuneatus was marked. King ('10), observing that very few fibers were shown in this tract by

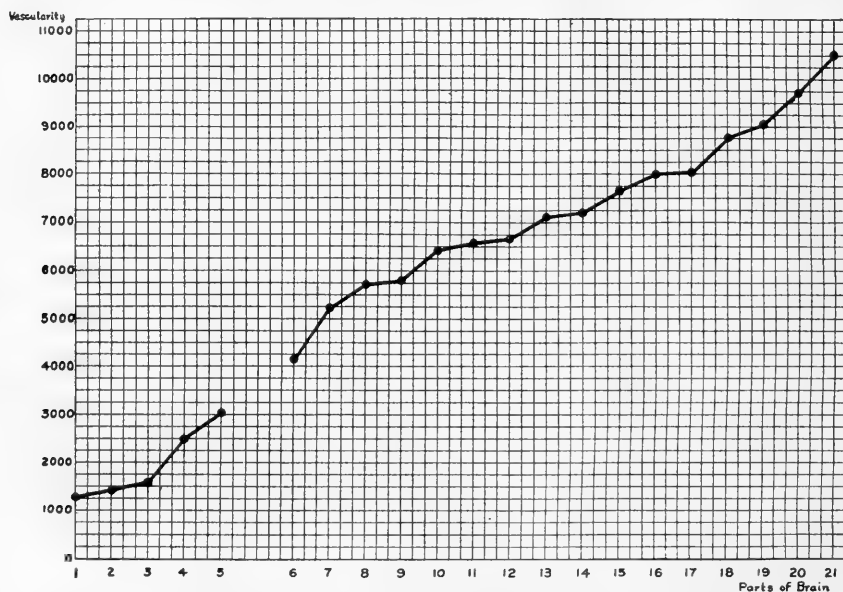


Chart 2 Graph showing the relative average vascularity of the regions studied.

Regions

- | | |
|---------------------------------------|------------------------------------------|
| 1, fasciculus cuneatus | 12, nucleus of Deiters |
| 2, ventral column | 13, molecular layer of cerebellar cortex |
| 3, lateral column | 14, dorsal horn; spinal cord |
| 4, pyramidal tract | 15, inferior olive |
| 5, fasciculus longitudinalis dorsalis | 16, superior olive |
| 6, substantia gelatinosa Rolandi | 17, chief sensory V nucleus |
| 7, nucleus motorius VII | 18, granule layer of cerebellar cortex |
| 8, nucleus XII | 19, nucleus dentatus |
| 9, nucleus motorius V | 20, chief vestibular nucleus |
| 10, ventral horn; spinal cord | 21, dorsal cochlear nucleus |
| 11, spinal V nucleus | |

the Marchi method, concluded that it is probably of only secondary functional importance in the rat. Ranson ('13), however, pointed out that King's results were in all likelihood due to the incomplete myelination of the tract, remarking that it was impossible to say how far this incomplete myelination might be indicative of functional insignificance. It is interesting to

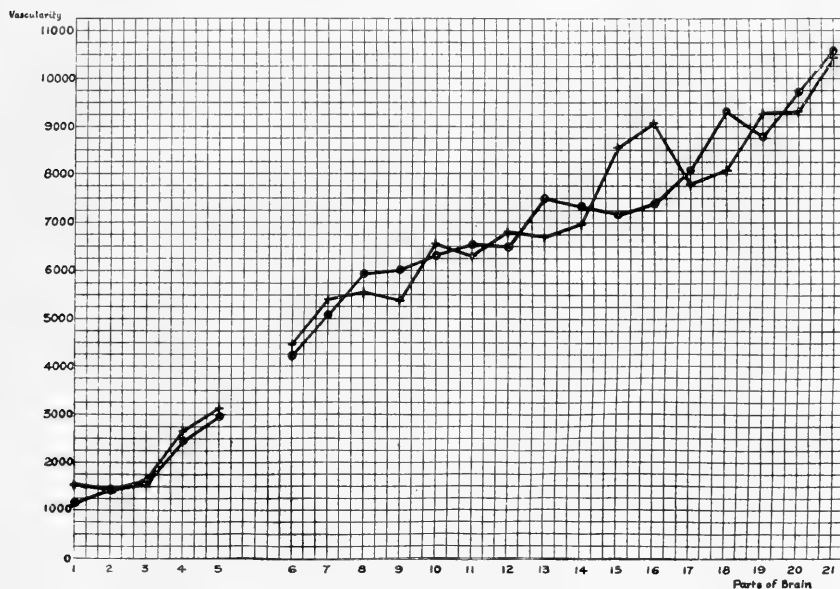


Chart 3 Graph showing the relation between the results obtained from Toronto and from The Wistar Institute animals. Toronto, ●; Wistar Institute, +. Numbers of regions as in chart 2.

To obviate any possible error in the construction of this graph arising from the disproportionate number of the two sexes, the figures for the four Toronto females were added together and divided by two. The quotient was then added to the figures for the two males and the whole divided by four. In this way the proportional value of the sexes became equal in each local group.

note the relatively high vascularity of this tract, suggesting great activity.

As six of the animals studied were obtained in Toronto, while the remaining four were from the standard colony of The Wistar Institute, it was thought advisable to group these two sets separately and compare the results. This is done in chart 3,

which shows that, in spite of irregularities, there is no evidence of any definite difference in the material as a whole between the two stocks.

Chart 4 shows the result of grouping the sexes separately and comparing them. It will be seen that the females show a distinct tendency to a richer vascular supply than the males, though the difference is neither very great nor very constant.

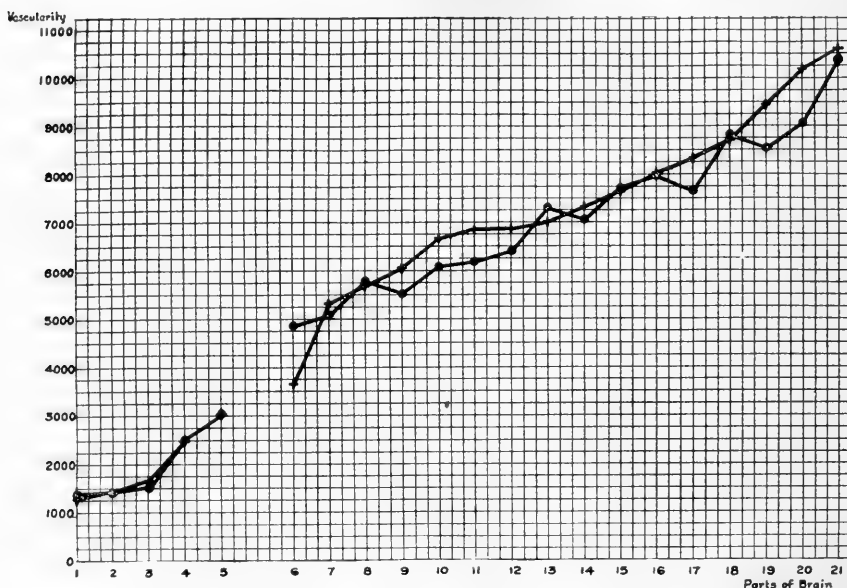


Chart 4 Graph showing the relation between the sexes in point of vascularity. Male, ●; female, +. Numbers of regions as in chart 2.

DISCUSSION

The attempt to interpret in terms of function the above anatomical observations leads us at once to the question of the importance of metabolic processes in nervous activity, and so to the even more fundamental question of the essential nature of the nervous impulse. Here we find ourselves in a sea of controversy, of varying opinions, and of contradictory evidence.

Hill and Nabarro ('95) found the oxygen consumption and the carbon-dioxide production in the brain to be low, while Alexander and Cserna ('13) found it to be very high—so high as to cast doubt upon their results (Bayliss, '18). The dark color of the blood in the cerebral veins, as Bayliss points out, suggests that there has been a very considerable consumption of oxygen, and Tashiro's experiments ('17 and earlier papers) lead him to believe that there is a rather high production of carbon dioxide in nerves and ganglia. MacArthur and Jones ('17), studying ground tissue, reached a similar conclusion regarding the central nervous system. There are also two recent papers by Moore ('18, '19) reporting a high acid production rate in the medulla, but a low one in the sciatic nerve. It may be noted in this connection that the total flow of blood through the brain in a given time is much greater than that through skeletal muscle and most other parts of the body (Jensen, '04). The most generally accepted view, however, appears to be that respiratory activity in nervous tissue is low.

Again, A. V. Hill's investigations ('12) are claimed to prove that the evolution of heat in nerve fibers is so small that any metabolism is doubtful, while recently Baglioni ('17) has shown that there is a distinct, though not high, production of heat in the central nervous system of the toad, which, moreover, bears a definite relation to functional activity.

Whether metabolic activity is high or low, there is still the question of what relation it bears to functional activity. "The problem of the essential physico-chemical nature of nerve conduction . . . is still regarded by most physiologists as unsolved, and apparently by many as insoluble" (Lillie, '18). Probably the most generally accepted opinion regarding the nervous impulse is that summed up by Bayliss as follows:

That it is a reversible, physico-chemical process, not associated with loss of material on account of metabolic reactions, is indicated by the following facts:

- Incapability of fatigue under normal conditions.
- Absence of formation of heat.
- Absence of decrement in wave.
- Low temperature coefficient of rate of conduction.
- No conclusive evidence of metabolism of any kind.

In keeping with this view, several theories as to the fundamental nature of nervous activity have been propounded. These generally postulate some form of electrolytic action as the essential process involved. Macdonald ('05) combines this conception with that of a colloidal suspension, about the particles of which the inorganic electrolytes are largely held in a 'masked' condition, from which stimulation causes them to be liberated. Macallum ('12), with experimental evidence in support of his contention similar to that of Macdonald, believes that the fundamental basis of nervous processes is surface tension.

Lillie ('18) maintains that a process parallel to nervous conduction is found in the wave of electrolytic activity which can be produced by similar means at the surface of a passive metal wire immersed in an acid solution. He is inclined "to regard the local bioelectric circuits accompanying normal cell activity as representing primarily some type of oxidation-reduction element," and his description of the process in the metal seems to involve the elimination of a certain, though probably minute, amount of waste matter as the impulse passes along.

On the other hand, certain experiments of Adrian ('18) suggest strongly that "the energy involved in the passage of the impulse is supplied locally from each point in the fiber through which the impulse passes, just as the explosion wave in a train of gunpowder is maintained by the energy in each part of the gunpowder as the wave reaches it and sets it alight." Johnston ('08) has brought forward certain purely anatomical evidence in favor of a similar view. Keith Lucas also supports this view, and after discussing the evidence (up to 1914) as to oxygen consumption and carbon-dioxide production in the nerve fiber, concludes: "However, the whole body of evidence is, I think, sufficient to justify the conclusion that the nerve uses oxygen and gives off carbon dioxide when it is conducting nervous impulses" (Lucas, '17). Adrian himself is less positive. He says (*loc. cit.*), regarding the nervous impulse: "Its nature is unknown, and the only direct accompaniment of it which can be detected with certainty is the electric response. . . . There is possibly an evolution of CO_2 at the same time, but this is still rather doubtful."

Lucas, then, and probably Adrian, basing their views upon certain characteristics observed in the conduction of the nervous impulse, seem to believe that it is chemical rather than physical in nature.

Direct evidence for this attitude is found in the work of Tashiro, who supports by strong experimental evidence, seconded by persuasive arguments against objections and contrary views, the thesis that every nervous process is accompanied by a definite change in carbon-dioxide production, indicative of a change in metabolic activity, and hence that such processes are essentially chemical in nature. It may be worth while to quote his conclusion (Tashiro, '17, p. 107):

Concerning the nature of the material basis of the nervous impulse we can only say that it appears to involve that part of the chemical transformations in protoplasm which result in the production of carbon dioxide. Farther than this we cannot go at present. But it is certain that it has a chemical basis. Whether it has also a physical basis, such as a change in state of the colloidal substratum of the nerve, or not, we cannot yet say. Who shall write the chemical reaction of the future, embracing not only the energy exchange, but the change in psychism as well?

(P. 108.) Three kinds of change occur, then, in our brains when the nerve impulses are passing — an electric change, a chemical change, and a psychical change. Which is the fundamental change?

A number of objections have been urged against Tashiro's work, which, moreover, seems in some particulars to involve considerable changes in views hitherto held concerning nervous action; and in spite of the strength of his evidence and arguments, the majority of physiologists appear to be disposed to reject it at the present time. It has received, however, rather striking support from the work of Baglioni (*loc. cit.*), who shows that, despite the earlier evidence to the contrary, there is a definite production of heat in the central nervous system during activity which seems to be parallel with the carbon-dioxide evolution demonstrated by Tashiro in peripheral nerves and ganglia. Moore, however (*loc. cit.*), has failed to confirm Tashiro's observations and is led to the conclusion that the processes underlying the nervous impulse do not produce carbon dioxide.

It is evident that while these questions are in their present unsettled condition, no final explanation can be formulated to account for the facts brought out in this study. We may consider briefly, however, the possible bearing upon them of some of the rival hypotheses.

If nervous activity is of a physical nature, involving no metabolic changes, then all the metabolism present will be the small amount required to maintain life in the protoplasm. So far as appears, this should not differ markedly from the rate of metabolism in the neuroglia, which likewise has little else to do, probably, than simply to perform the ordinary vegetative functions and passively to occupy its place. There will also be a certain amount of reserve energy available for growth or repair, a reserve which one may assume to be of rather more importance to the neuroglia than to the nervous elements themselves, and which probably will not differ very greatly in different regions.

What, then, is the significance of the differences in vascularity observed in various centers? With regard to the rather large difference existing in general between gray and white matter, this very condition has been brought forward as an argument in support of the contention that metabolism is practically absent in nerve fibers, and it certainly seems to agree with it. The capillaries present in the white matter will in this case be concerned almost entirely with the needs of the neuroglia. The richer blood supply in the tract which is largely unmyelinated may be due to the presence of a greater proportion of neuroglia surrounding a larger number of much thinner nerve fibers in a unit volume, space not being occupied by the large bulk of the myelin sheaths. The myelinated fasciculus longitudinalis dorsalis, however, is much richer than is this (pyramidal) tract, so that the significance of the facts is not clear. The observation that about 50 per cent of the white matter is composed of myelin (Donaldson and Hoke, '05; Greenman, '13, '17; Koch and Koch, '17) would in itself explain the fact that the vascularity of the more poorly supplied gray regions is in the neighborhood of twice as great as that of the white matter of the cord.

The nourishment of the nerve fibers, according to what seems to be the fairly generally accepted view, is derived very largely, if not entirely, from the cell body. It is difficult to believe, however, that a fiber a meter long can be nourished entirely by the activity of a single cell body situated at one end of it, though the ordinary conception of the functions of the nucleus would lead one to expect metabolism as a whole to be more rapid, and perhaps more important in some respects, in that part of the neurone which contains it. Certain authors state that the myelin sheath is concerned in the nutrition of the axone, and if this be true, it is easy to see that myelination may enable a tract to carry on its functions satisfactorily with a much poorer blood supply than would otherwise be needed, if activity is not constant. The arguments in favor of this view are summed up by Mathews ('16). Moreover, the neuroglia itself may perhaps act as an intermediary between the blood and the nervous elements. Achúcarro ('15) attributes to the neuroglia considerable functional activity "as an interstitial gland which acts on the nerve elements and on the blood, contributing by means of special hormones to the endocrinic harmony of the organism." Cajal also believes that the neuroglia bears a nutritive relation to the nerve cells, and acts as a chemical rejuvenator toward them.

It is to be noted that the susceptibility of the reflex arc to anaemia, lack of oxygen, drugs, etc., is much greater than that of the nerve trunk, which shows that the blood supply is actually of greater direct physiological importance to some part of the neurone which lies in the gray matter than to the axone (Sherrington, '06).

The difficulty is perhaps even greater, and the problem is of even more immediate interest, when we come to consider the differences in the vascularity of the various centers of gray matter. If the functional activities of the neurones involve no metabolic changes, and the only chemical processes occurring are those incident to the relatively small amount of assimilation and respiration necessary to maintain passive life, what is the meaning of the fact that, for example, the blood supply of the dorsal cochlear nucleus is from nearly one and a half times to twice as

great as that of the ventral cornu of the spinal cord or that of the hypoglossal nucleus? While there is no proof that such a difference in vascularity necessarily implies a corresponding difference in metabolic activity in the regions concerned, that assumption, as made by Aby, seems to be the only reasonable explanation (in so far as it is an explanation) of the facts. Moreover, it is quite in accord with what is known of the blood supply to other tissues (e.g., Krogh, '19). The considerable individual variations observed suggest that the correspondence is not very exact, and no doubt each region is supplied somewhat in excess of its normal requirements. It is difficult to believe, however, that there would be any constant and considerable differences, such as have been demonstrated, if the quantity of materials used and of waste products given off were about the same in the various parts compared. Also, the metabolic differences must really be considerable and regular, as the vascularity suggests, for any small or occasional variations in chemical activity could probably be satisfactorily dealt with by the cerebrospinal fluid (Halliburton, '16; Weed, '17) and, perhaps, the neuroglia, as suggested above. The observation of Hatai ('17), that "the relation between metabolic products and active cell substance is quantitatively similar in all parts of the central nervous system and in both parts of the neurone," seems to indicate that much more of these substances must be produced in certain parts, as otherwise the much greater blood supply in these parts would cause them to be much poorer in residual products of metabolism, which are what Hatai measured. His conclusion, that the quantitative relation which he demonstrates between the active cell substance and the metabolites indicates "an equality in activity so far as nitrogen metabolism is concerned," does not appear to agree with the facts regarding the blood supply which are recorded above.

If the metabolic processes are not much more active in the richly vascular regions than in the poor ones, we might expect that the cells in the latter would probably be the first to succumb to anaemia. This, however, is by no means the case. The small pyramidal cells in the cortex survive only eight minutes,

while the cells of the spinal cord survive for three-quarters of an hour or more (Cannon and Burkett, '13).

If it be assumed that the observed differences in vascularity are indicative of corresponding differences in rate of metabolism, the latter may be accounted for in either of two ways. Provided that the rate of metabolism is markedly greater in the nervous elements than in the neuroglia (for which there seems to be no particular reason if nervous processes are purely physical in nature), this difference may mean simply that the nerve cells are packed together more closely in one case than in another—that there is a greater volume of nervous protoplasm present in a unit volume of the tissue. This is practically the view stated by Obersteiner (*loc. cit.*), who says: “the richer any region is in nerve cells the closer is the capillary network which supplies it.” There can be no doubt that this factor plays a part, some of the centers having a considerable admixture of white matter, and the cells certainly being more numerous in some parts than in others. The facts, however, do not seem to be adequately met by this view, and we are impelled to look about for something else.

The only other explanation which offers itself appears to be that the difference in rate of metabolism deduced from the difference in vascularity corresponds to a difference in functional activity. This implies the acceptance of the view that nervous processes involve metabolic changes, for which belief, as we have seen, the physiological evidence is as yet inconclusive. Even if it be true, one may well wonder why the activity of sensory nuclei should be greater than that of motor centers and why there should be such marked differences within each of these groups. Evidently, our knowledge of nervous processes will have to advance a long way before these matters can be fully understood. It may, however, be suggested, with all due diffidence, that sensory centers are in more or less constant receipt of stimulation, while the activity of motor centers is rather intermittent. On the other hand, it may be pointed out that the motor centers which are concerned with muscle tone must be in a state of more or less continuous excitation.

There have also been recorded differences in the manner of growth of certain sensory and motor cells, which seem to be of such a nature as to demand a larger blood supply for the former. These differences have been described by Donaldson and Nagasaka ('18), who observed that the spinal ganglion cells grow with the growth of the body, each enlarging "nearly in proportion to its entire fibers, but less rapidly than the corresponding axes;" while in the case of the large spinal-cord cell bodies, "the enlargement of the axon to meet the requirements of the increased muscle mass to be innervated is not accompanied by any notable increase in the size or internal arrangements of the cell." The growth of the spinal ganglion cell "is considered as an adaptation for maintaining the sensory discrimination despite the extension of the area supplied by a single neuron."

Finally, one cannot altogether overlook the existence of what are generally designated 'psychic' processes, whatever these may really be, and whatever may be the nature of their relation to physiological activity. Von Monakow ('16) believes "that the material basis of the sentiments ought to be regarded as chemical." Cannon and Crile both claim to have found evidence of endocrine activity in emotions, and Achúcarro believes that the neuroglia may be involved in such processes. " 'Evidently, then,' as Lagaro remarks, 'the nervous system and the endocrinic glands act as one under certain circumstances and constitute the basis of many changes in normal psychic life.' " (Orr and Rows, '18). Mott ('14) states quite decidedly that the physiological basis of all mental activity, whether simple or complex, is a group of biochemical processes involving oxidation and hence absolutely dependent upon the blood supply. Thus it is at least possible that the vascular differences recorded above may be in part related to 'psychic' activities in which the respective centers are concerned.

SUMMARY

No exact quantitative comparison of the vascularity of different parts of the central nervous system has been published hitherto, and the present study is an attempt partly to fill this gap. To that end, anatomical measurements of the capillary richness have been made in twenty-one regions arbitrarily selected in the spinal cord, medulla oblongata, and cerebellum of the albino rat, and the values obtained and their ratios are presented in tabular and graphic form. It is not claimed that these values represent the absolute vascularity of the parts concerned, but it is believed that they do show in a fairly reliable manner the relative richness of the capillary supply. The results may be summarized as follows:

1. The gray matter is much more richly supplied with capillaries than is the white matter, the poorest part of the gray being nearly half as rich again as the richest part of the white among the regions studied.

2. All parts of the white matter are not equally vascular, the pyramidal tract, the richest part in the spinal cord, being about twice as rich as the fasciculus cuneatus, while the fasciculus longitudinalis dorsalis in the medulla is still richer.

3. The gray centers can be sharply divided into two groups, the motor nuclei and the sensory and correlation centers, of which the latter are richer than the former. Though the richest motor region (ventral cornu) is but little poorer than the poorest sensory one (spinal V nucleus), the two groups do not overlap in the case of those regions studied, except in a few individuals. The substantia gelatinosa Rolandi of the spinal cord is the only part which does not conform with this statement.

4. The richest of the centers observed is the dorsal cochlear nucleus, which is more than half as rich again as the ventral cornu, about two and a half times as rich as the substantia gelatinosa Rolandi (the poorest gray region), and eight times as rich as the fasciculus cuneatus.

5. Great individual variations occur, and the two sexes do not seem to show any constant difference.

6. The interpretation of the functional significance of the anatomical data recorded depends upon the unsolved problems of the fundamental nature of nervous processes and their relation to metabolic activity. The bearing of various theories regarding these upon the present investigation is discussed.

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Resumen por el autor, J. M. D. Olmsted,
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El nervio como influencia formativa en el desarrollo de los botones
gustativos.

En todos los estados de la regeneración de los extremos de las barbillas del pez *Amiurus nebulosus*, el nervio y el cartílago ocupan prácticamente toda la distancia entre la porción no extirpada y la membrana basal de la epidermis del extremo terminal de la barbilla regenerada. Las porciones regeneradas de escaso tamaño, aun cuando presentan cartílago y el nervio, no presentan vestigios de botones gustativos. La formación de las papilas dérmicas, que preceden invariablemente a los botones gustativos, tiene lugar en la base de las porciones regeneradas mas largas, como si la capa germinativa de la epidermis se hundiese en ciertas zonas a consecuencia de la penetración de una pequeña rama procedente del tronco nervioso. Puesto que los botones gustativos degeneran en las barbillas en las cuales se ha cortado el nervio, y reaparecen con la regeneración de éste, y además, puesto que el nervio aparece en la región correspondiente antes de que haya indicio alguno del desarrollo de un botón gustativo, la presencia del nervio puede considerarse como el factor causal en la formación de los botones gustativos.

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THE NERVE AS A FORMATIVE INFLUENCE IN THE DEVELOPMENT OF TASTE-BUDS¹

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INTRODUCTION

The influence of one organ upon the development of another is a fundamental problem in morphogenesis. Embryologists have rather taken for granted that the differentiation of specialized organs, such as the transformation of epithelial cells into taste-buds, is due to the growth of the appropriate nerve into the region concerned. Hermann ('84), who first described the development of taste-buds in the dog, seems to hold this view, though he states that one sees the embryonic nerve beneath the germinative layer of the epidermis after the dermal papilla appears. The nerve then passes up to the forming taste-bud, and finally the characteristic spindle-shaped taste-cells become differentiated.

Marchand ('02), who studied the developing papillae in the human foetus, states: "Vers le cinquième mois, certaines cellules de la couche génératrice commencent alors à se différencier pour donner naissance aux borgeous gustatifs. Les nerfs gustatifs qui commandent la différenciation sont arrivés au contact de l'épithélium."

Landacre ('07), in his paper on the place of origin and distribution of taste-buds in *Amiurus melas*, says, in regard to the question whether the taste-buds appear fortuitously and are later connected with their gustatory nerves or whether the nerve fibers take the initiative and produce a bud on the surface, that the evidence is much more in accord with the latter view. His best proof is that the smaller subdivisions of the groups of taste-buds are determined by the number of nerves supplying these subdivisions, and that buds and nerves appear practically simultaneously. "The assumption . . . that the appearance of

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the taste-bud indicates the time at which the nerve supplying it reaches the surface needs verification for taste-buds in *Amiurus*."

There are two cases in which it seems to have been proved that differentiation of sense organs is dependent upon the nerve, namely, the formation of the tactile corpuscles of Merkel (Szymonowicz, '95) and of Grandry's and Herbst's corpuscles (Szymonowicz, '96).

The growth of such organs as teeth, however, is claimed to be absolutely independent of the nervous system (Moral and Hoseman, '19). But the nerve does exert a regulating influence, either increasing or decreasing the rate of growth, or causing changes in color of the tooth.

In another paper (to appear shortly) I have described the degeneration of taste-buds which occurs after severing the branches of the seventh cranial nerve leading to the barbels of the catfish, *Amiurus nebulosus*, and also the reappearance of taste-buds directly attendant upon the regeneration of the nerve. The present paper affords additional evidence that the presence of the nerve is the formative influence in the development of taste-buds.

MATERIALS AND METHODS

When the end of a barbel (0.5 to 1 cm.) of *Amiurus* is cut off, sufficient regeneration takes place under normal circumstances to become evident to the eye at the end of two weeks. The regenerated portion appears as a colorless finger-like projection, having less than half the diameter of the stump from which it springs. When prepared with Mallory's phosphotungstic haematoxylin, according to the directions given on page 369 of Mallory and Wright's "Pathological Technique," the cartilage, pigment cells, and connective tissue stain a brilliant red; the nuclei of epidermal cells and of the nervous tissue stain a brilliant blue; while the nerve fibrils and cytoplasm of the epidermis, especially the cytoplasm of the sense cells of the taste-buds, take on a characteristic lilac hue.

RESULTS

Such preparations show that regeneration is more rapid in the region near the old stump of cartilage. It is rapid growth in this region that causes the finger-like appearance of the new part.

A column of large hyaline cells extends from the old cartilage nearly to the basement membrane of the epidermis at the very tip of the new portion of the barbel. In the younger specimen with a regenerated end, i.e., 2 to 3 mm. in length, these precartilaginous cells stain a light blue, but in later stages of regeneration they take the typical brilliant red of the old cartilage. These cells are readily distinguishable by their form and staining properties. Along the anterior border of this column of precartilaginous cells is always seen, even in the shortest regenerated pieces, a small amount of fibrous material which stains the characteristic lilac hue, and which when traced to its origin is always found to be continuous with the old nerve trunk. Certain sagittal sections bring out this relationship most favorably in a single section, and the connection can be readily traced in a series of transverse cross-sections. These fibers extend in bundles between the rod of precartilaginous tissue and the generative layer of the epidermis throughout practically the whole length of the regenerated tip of any barbel.

When the regenerated end of a barbel is less than 2 or 3 mm. in length, the generative layer of the epidermis extends in a smooth unbroken sheet around the entire new end. Both transverse and sagittal sections show this unbroken line, and yet the nerve extends practically throughout the length of the new piece. But when a length of 3 to 4 mm. is reached, one can see several indentations in the generative layer. These appear first in the region near the junction between the old and new tissue, and particularly along the anterior border of the new nerve. These indentations are the beginnings of the dermal papillae, the invariable forerunners of the taste-buds. Each papilla is filled with a small bundle of nerve fibers which stands out from the nerve trunk like a small button, almost as if they had exerted such force in their growth out from the nerve that they had indented the generative layer at that spot.

Later stages of regeneration showed the presence of fully developed taste-buds along the whole length of the regenerated end, mainly concentrated, however, along the edge nearest the nerve. The development of mature taste-buds after the formation of the dermal papillae is to be described in a later paper.

CONCLUSIONS

It is evident, therefore, that the growth of the nerve into the appropriate region precedes the appearance of taste-buds, and that the formation of dermal papillae, the immediate forerunners of the taste-buds, is most intimately connected with the growth into it of the particular branch of the nerve trunk which is to innervate it.

SUMMARY

1. The nerve and cartilage in all stages of regenerating ends of barbels of the catfish, *Amiurus nebulosus*, extend practically the complete distance from the old stump to the basement membrane of the epidermis at the very tip.

2. Short regenerated pieces, though possessing cartilage and nerve, show no trace of taste-buds.

3. The formation of dermal papillae, the invariable forerunners of taste-buds, takes place at the base of longer regenerated pieces as if the germinative layer of the epidermis were indented by the growth into it of a small branch from the nerve trunk.

4. Since taste-buds degenerate in a barbel whose nerve is cut and reappear when the nerve regenerates, and since the nerve appears in the appropriate region before there is any evidence of a developing taste-bud, the presence of the nerve may be said to be the causative factor in the formation of taste-buds.

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